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(54) **HUMANIZED ANTI-CD70 BINDING AGENTS AND USES THEREOF**

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(57) **ABSTRACT**

Disclosed are CD70 binding agents, such as humanized anti-CD70 antibodies and fragments and derivatives, that exert a cytotoxic, cytostatic or immunomodulatory on CD70 expressing cells, as well as pharmaceutical compositions and kits comprising the antibody, fragment or derivative. Also disclosed are methods for the treatment of CD70-expressing cancers and immunological disorders, comprising administering to a subject the CD70 binding agents or pharmaceutical compositions.

Specification includes a Sequence Listing.

FIG. 1

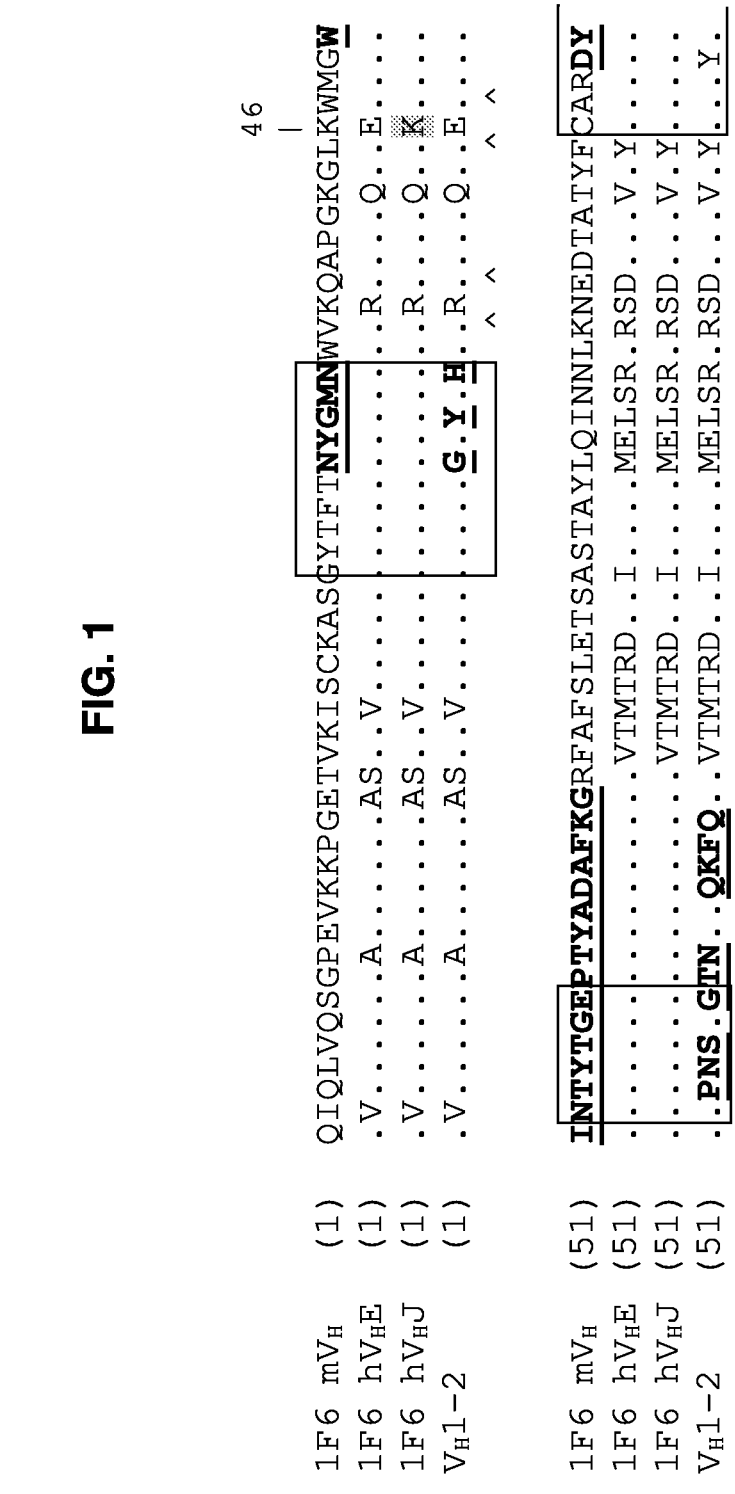

46
1F6 mVH (1) QIQLVQSGPEVKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGW
1F6 hVHE (1) .V.....A.....AS..V.....R....Q..E....
1F6 hVHJ (1) .V.....A.....AS..V.....R....Q..K....
VH1-2 (1) .V.....A.....AS..V.....G.Y.H..R....Q..E....
1F6 mVH (51) INTYTGEPTYADAFKGRFAFSLETSASTAYLQINNLKNEDTATYFCARDY
1F6 hVHE (51) .....VTMTRD..I....MELSR.RSD..V.Y....
1F6 hVHJ (51) .....VTMTRD..I....MELSR.RSD..V.Y....
VH1-2 (51) .PNS.GTN..QKFQ..VTMTRD..I....MELSR.RSD..V.Y..Y.
1F6 mVH (101) GDYGMDYWGQGTSVTVSS
1F6 hVHE (101) .....T.....
1F6 hVHJ (101) .....T.....
JH6 YY....V.....T.....

FIG. 2

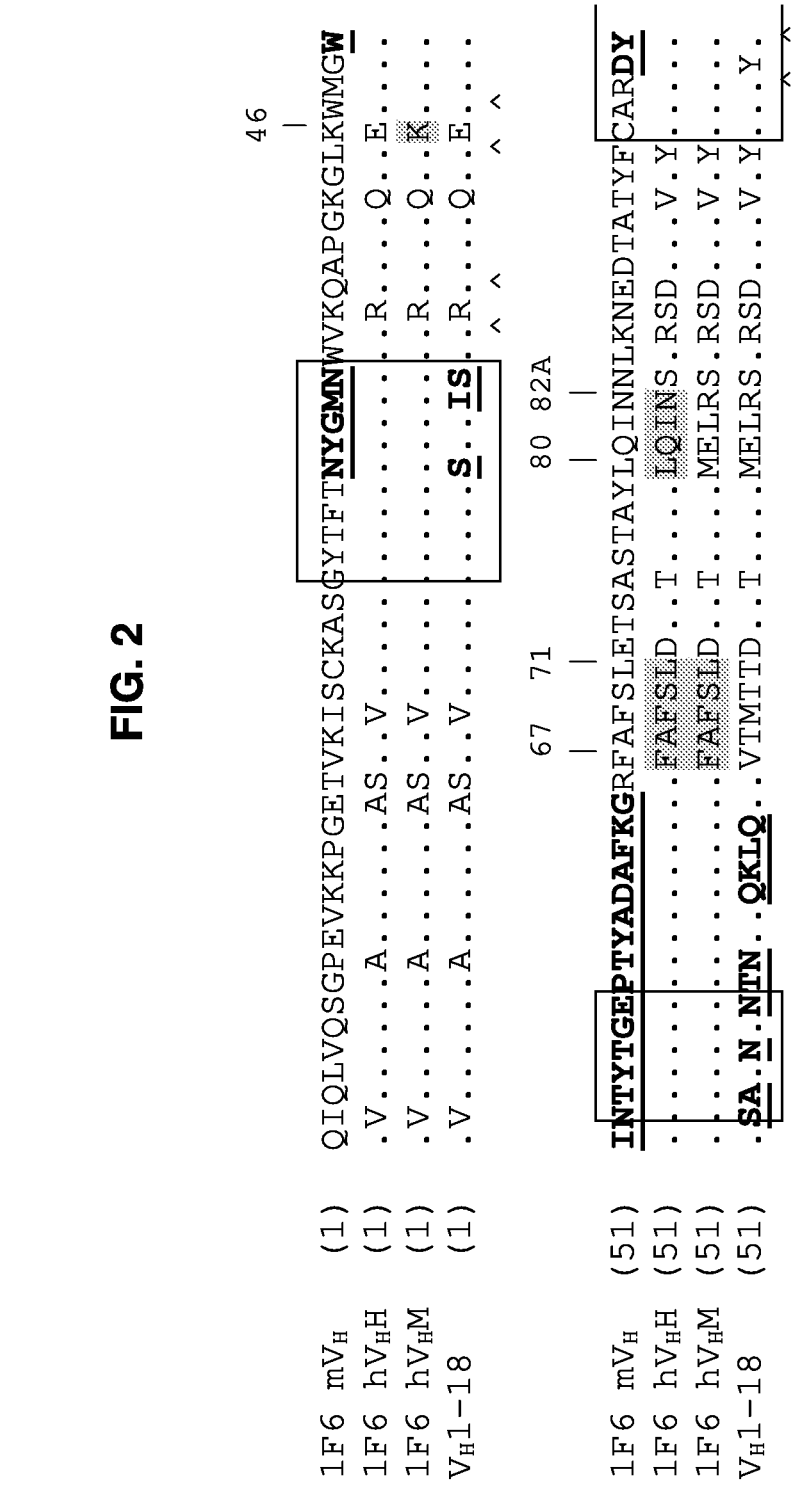
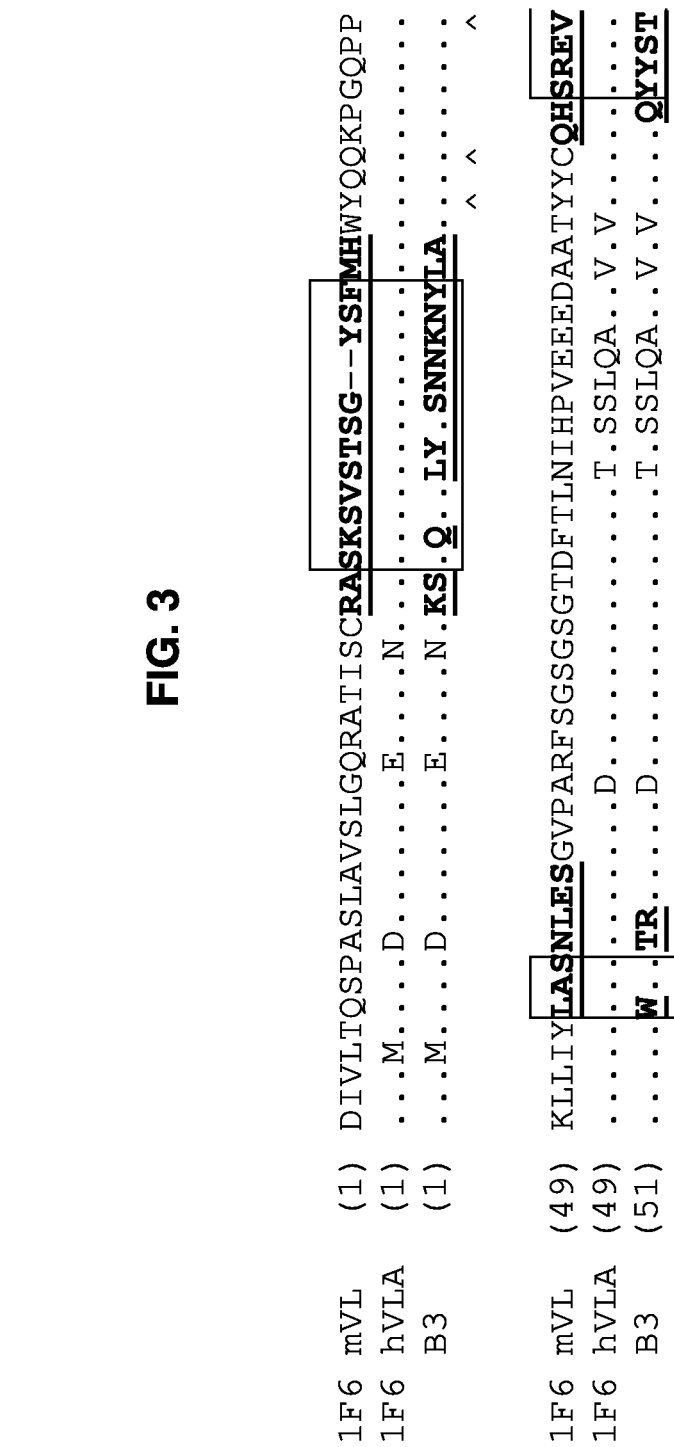

46
1F6 mVH (1) QIQLVQSGPEVKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGW
1F6 hVHH (1) .V.....A.....AS..V.....R....Q..E....
1F6 hVHM (1) .V.....A.....AS..V.....R....Q..K....
VH1-18 (1) .V.....A.....AS..V.....S..IS..R....Q..E....
67 71 80 82A
1F6 mVH (51) INTYTGEPTYADAFKGRFAFSLETSASTAYLQINNLKNEDTATYFCARDY
1F6 hVHH (51) .....FAFSLD..T....LQINS.RSD...V.Y.....
1F6 hVHM (51) .....FAFSLD..T....MELRS.RSD...V.Y.....
VH1-18 (51) .SA.N.NTN..QKLQ..VTMTTD..T....MELRS.RSD...V.Y...Y.
1F6 mVH (101) GDYGMDYWGQGTSVTVSS
1F6 hVHH (101) .....T.....
1F6 hVHM (101) .....T.....
JH6 YY....V.....T.....

FIG. 3


1F6 mVL (1) DIVLTQSPASLAVSLGQRATISCRASKSVSTSG--YSFMHWYQQKPGQPP
1F6 hVLA (1) ...M....D.....E....N...
B3 (1) ...M....D.....E....N.KS.Q..LY.SNNKNYLA.....
^ ^ ^
1F6 mVL (49) KLLIYLASNLESGVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHSREV
1F6 hVLA (49) .....D.....T.SSLQA..V.V.....
B3 (51) .....D.....T.SSLQA..V.V...QYYST
^ ^
1F6 mVL (99) PWTFGGGTKLEIKR
1F6 hVLA (99) .....Q...V....
B3/JK-1 (101) .....Q...V...

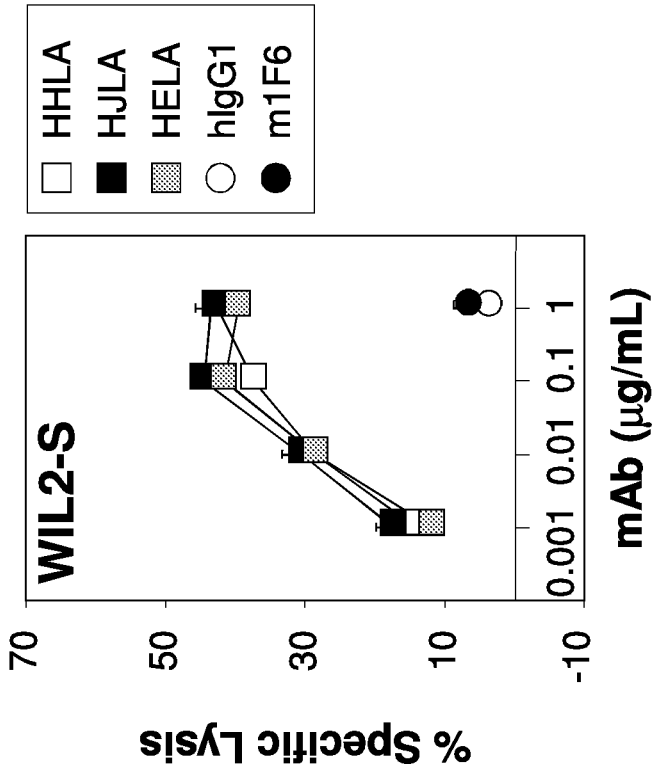

HHLA
HJLA
HELA
hIgG1
m1F6
WIL2-S
% Specific Lysis
70
50
30
10
-10
0.001
0.01
0.1
1
mAb (μg/mL)

FIG. 4A

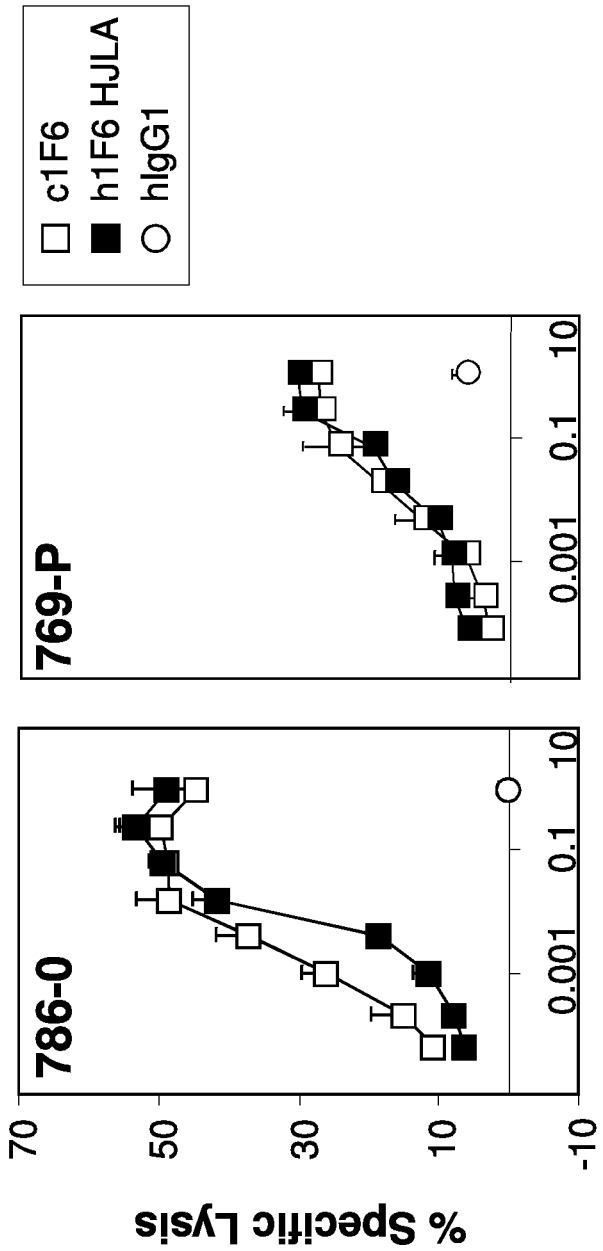
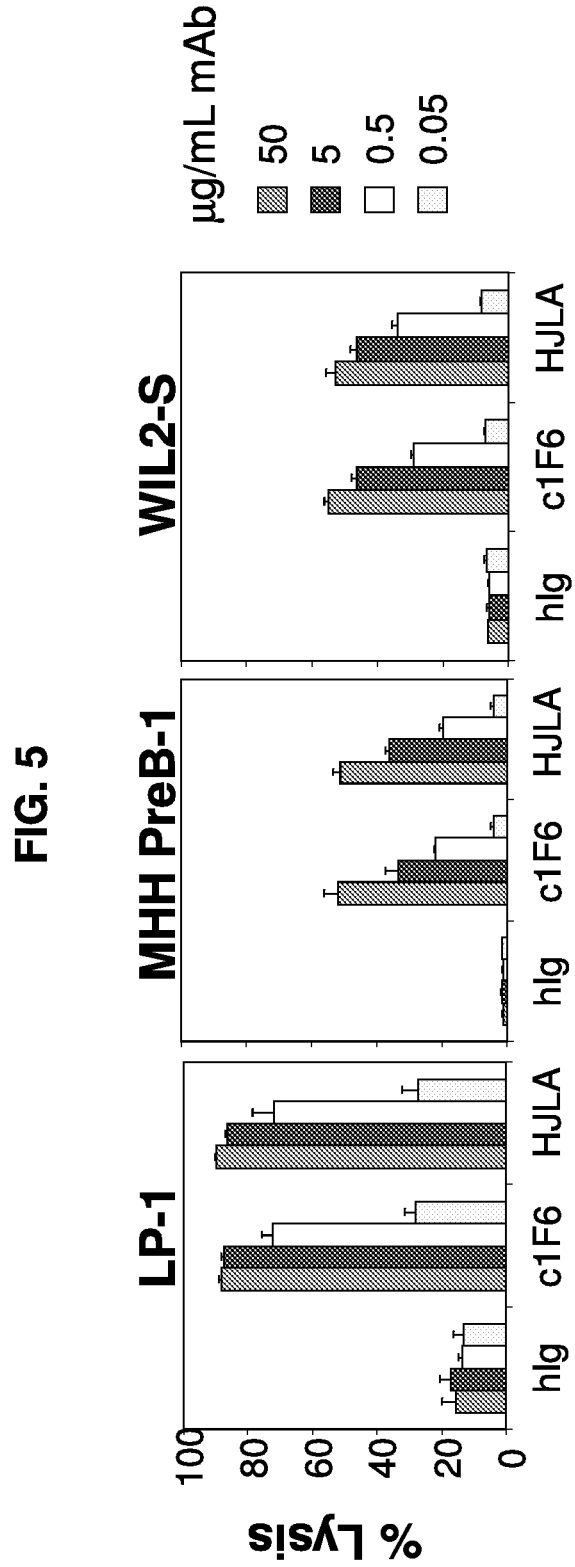

c1F6
h1F6 HJLA
hIgG1
786-0
769-P
% Specific Lysis
70
50
30
10
-10
1E-05
0.001
0.1
10
mAb (µg/mL)

FIG. 4B

FIG. 5


% Lysis
100
80
60
40
20
0
LP-1
MHH PreB-1
WIL2-S
hIg
c1F6
HJLA
µg/mL mAb
50
5
0.5
0.05

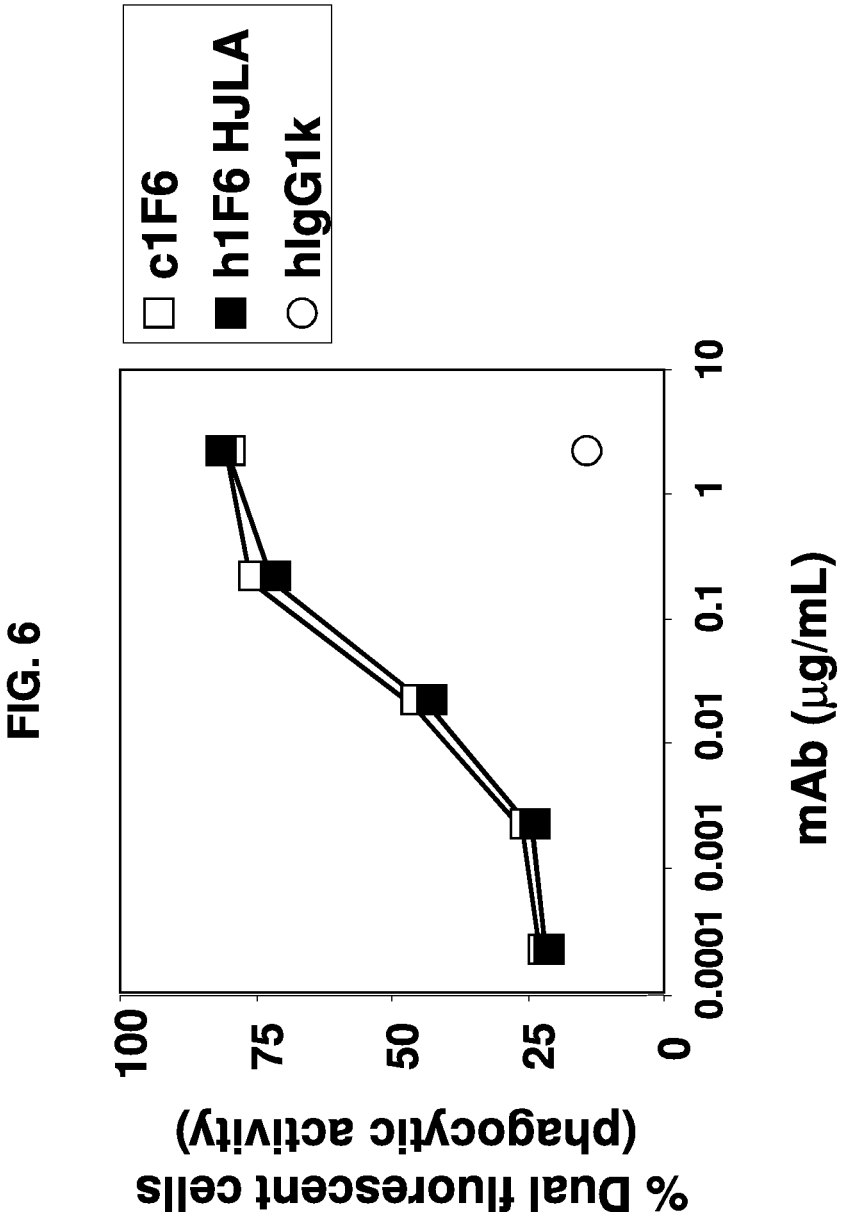

c1F6
h1F6 HJLA
hIgG1k
% Dual fluorescent cells
(phagocytic activity)
100
75
50
25
0
0.0001 0.001 0.01 0.1 1 10
mAb (μg/mL)

FIG. 6

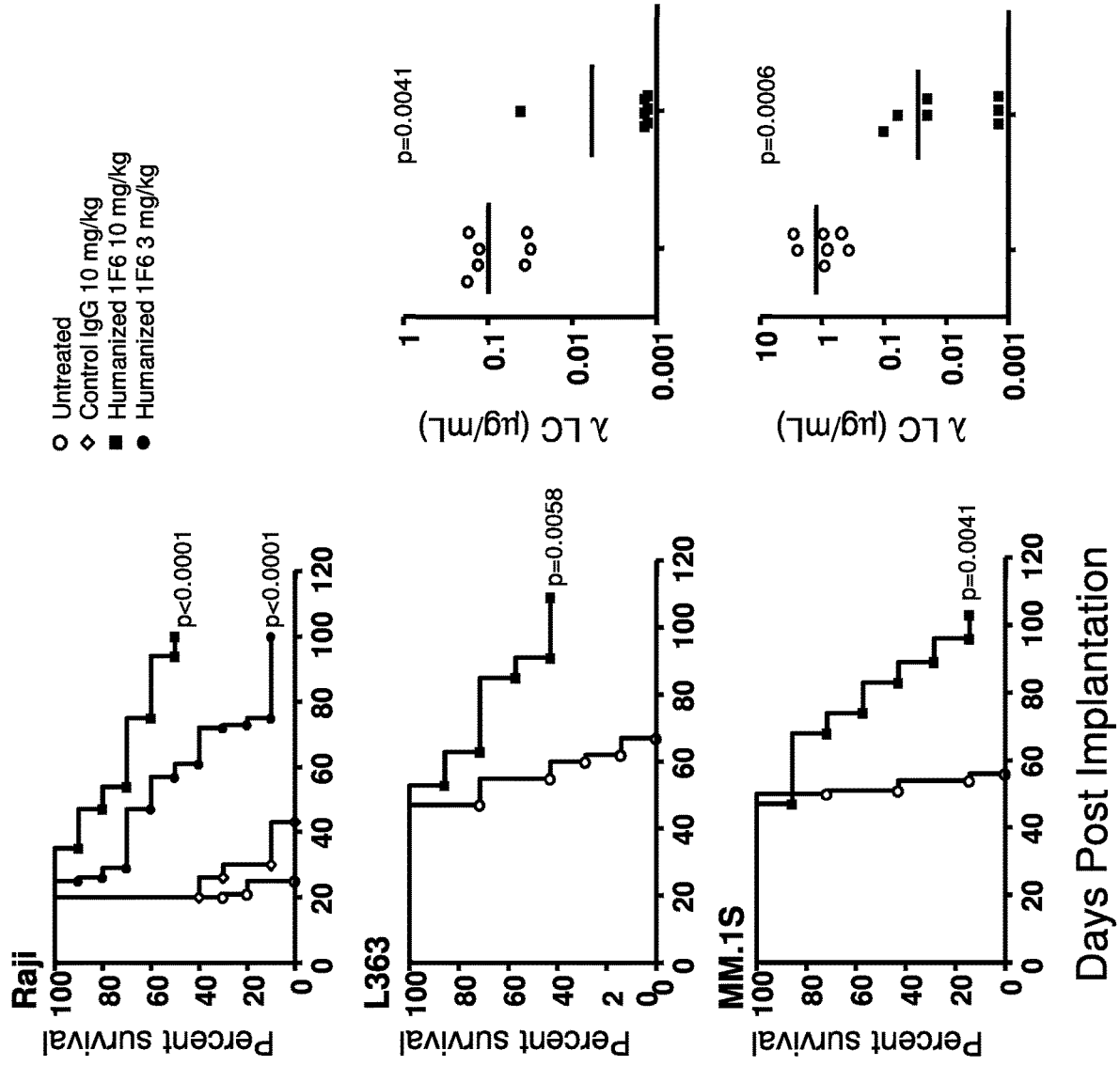


FIG. 7A

FIG. 7B

FIG. 7C

FIG. 8A

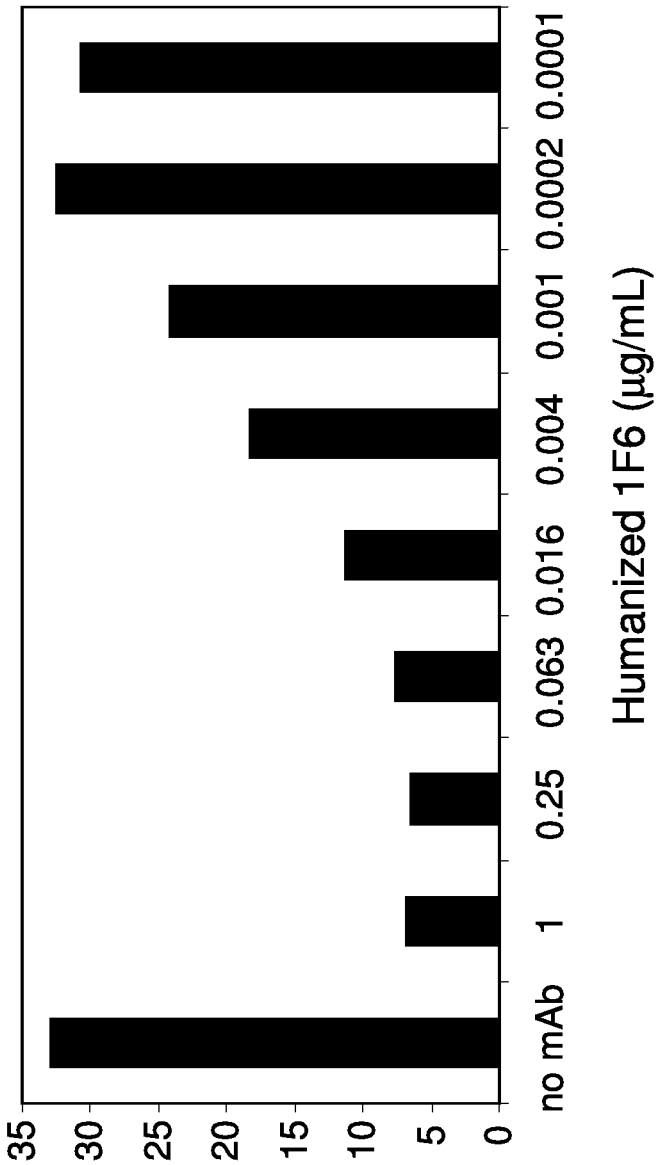

CD8+Vβ17+ (%)
35
30
25
20
15
10
5
0
no mAb
1
0.25
0.063
0.016
0.004
0.001
0.0002
0.0001
Humanized 1F6 (µg/mL)

FIG. 8B

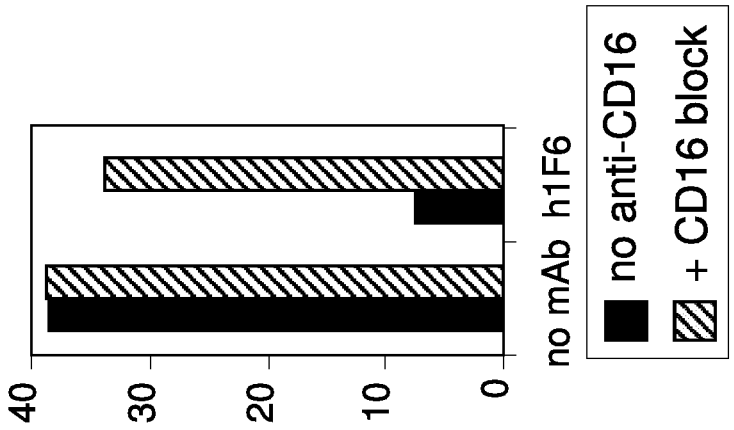

40
30
20
10
0
no mAb
h1F6
no anti-CD16
+ CD16 block

FIG. 9

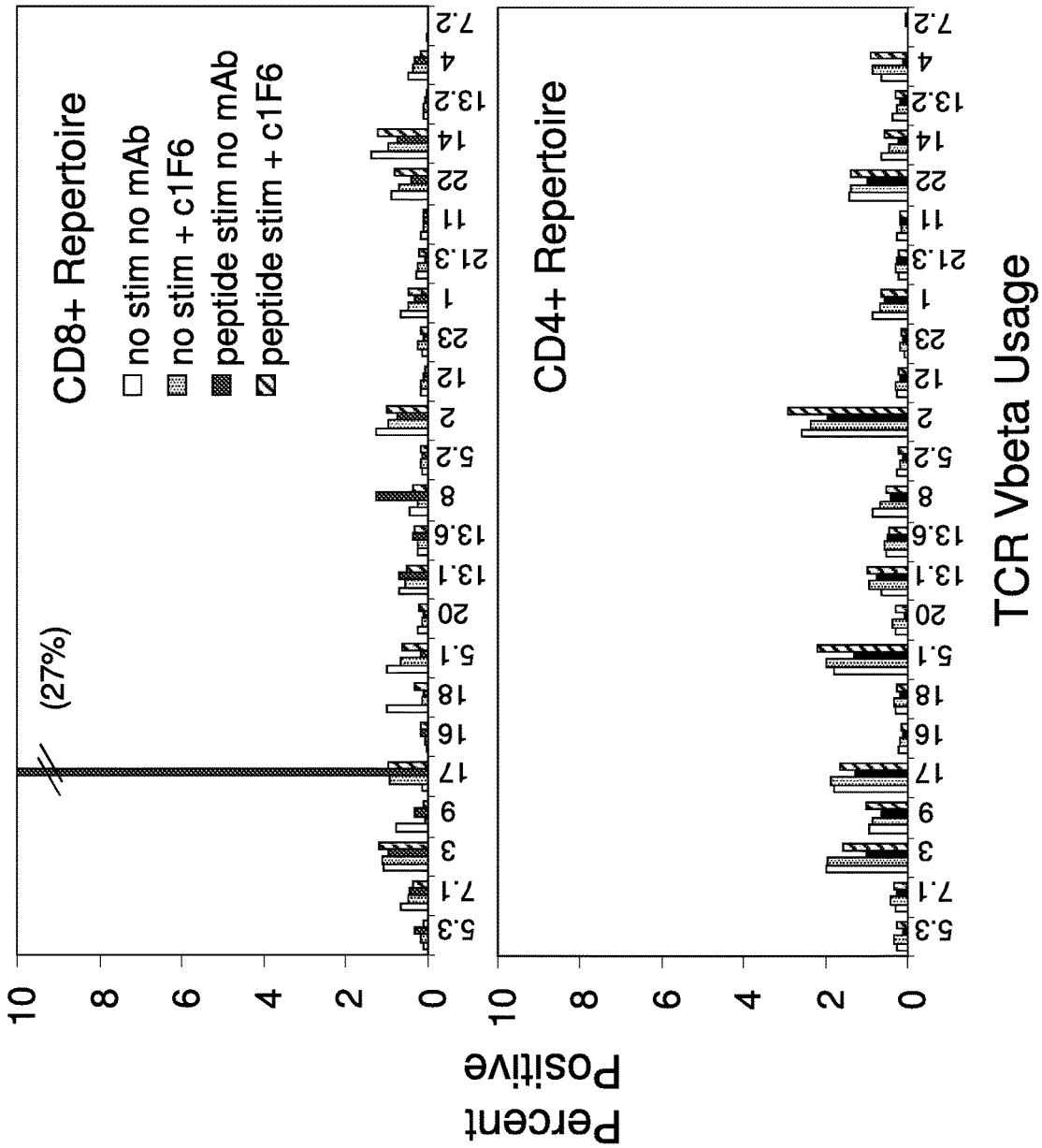

CD8+ Repertoire
no stim no mAb
no stim + c1F6
peptide stim no mAb
peptide stim + c1F6
(27%)
CD4+ Repertoire
Percent Positive
TCR Vbeta Usage
0
2
4
6
8
10
5.3
7.1
3
9
17
16
18
5.1
20
13.1
13.6
8
5.2
2
12
23
1
21.3
11
22
14
13.2
4
7.2

FIG. 10A

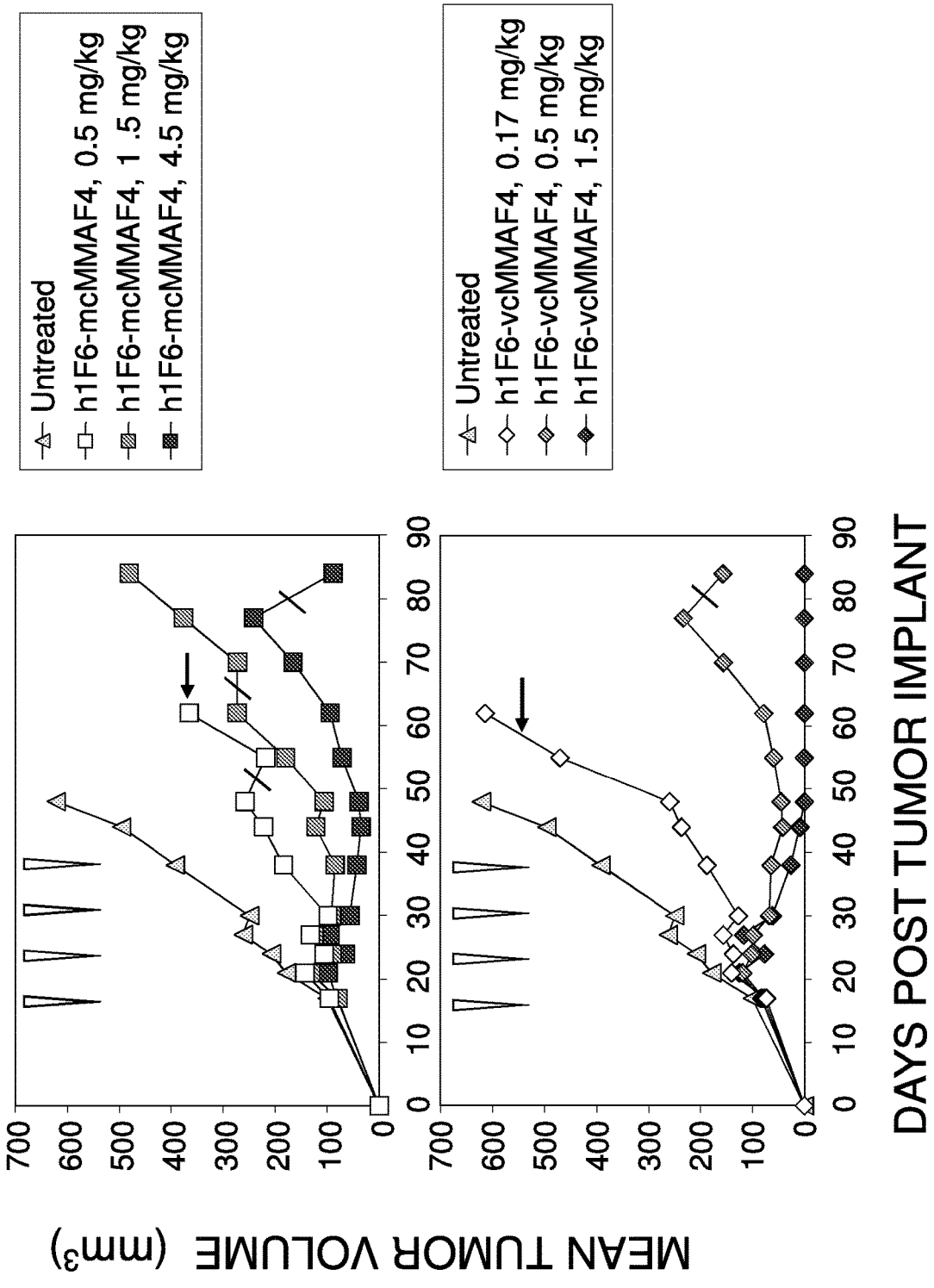

Untreated
h1F6-mcMMAF4, 0.5 mg/kg
h1F6-mcMMAF4, 1 .5 mg/kg
h1F6-mcMMAF4, 4.5 mg/kg
Untreated
h1F6-vcMMAF4, 0.17 mg/kg
h1F6-vcMMAF4, 0.5 mg/kg
h1F6-vcMMAF4, 1.5 mg/kg
MEAN TUMOR VOLUME (mm3)
0
100
200
300
400
500
600
700
0 10 20 30 40 50 60 70 80 90
DAYS POST TUMOR IMPLANT

FIG. 10B

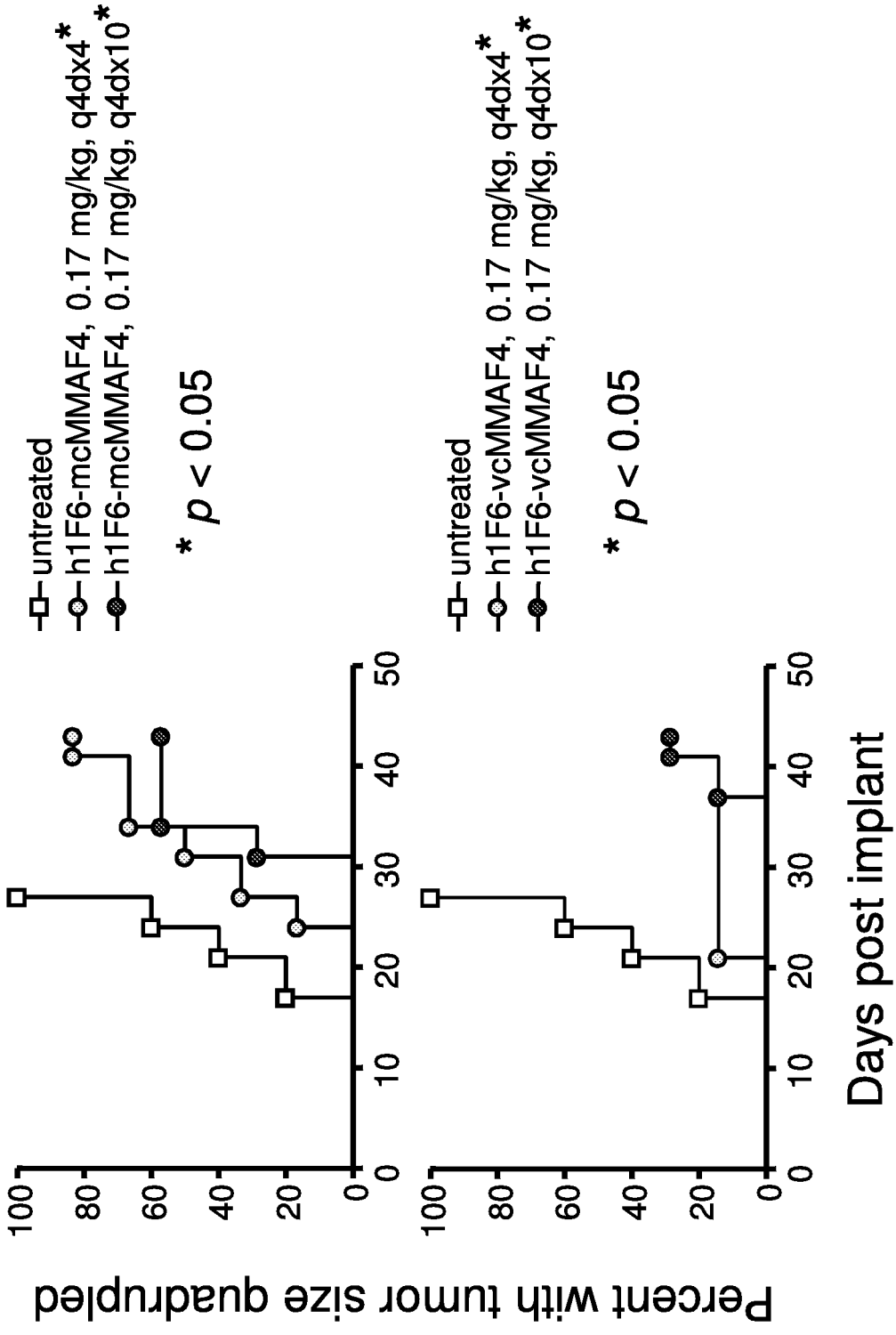
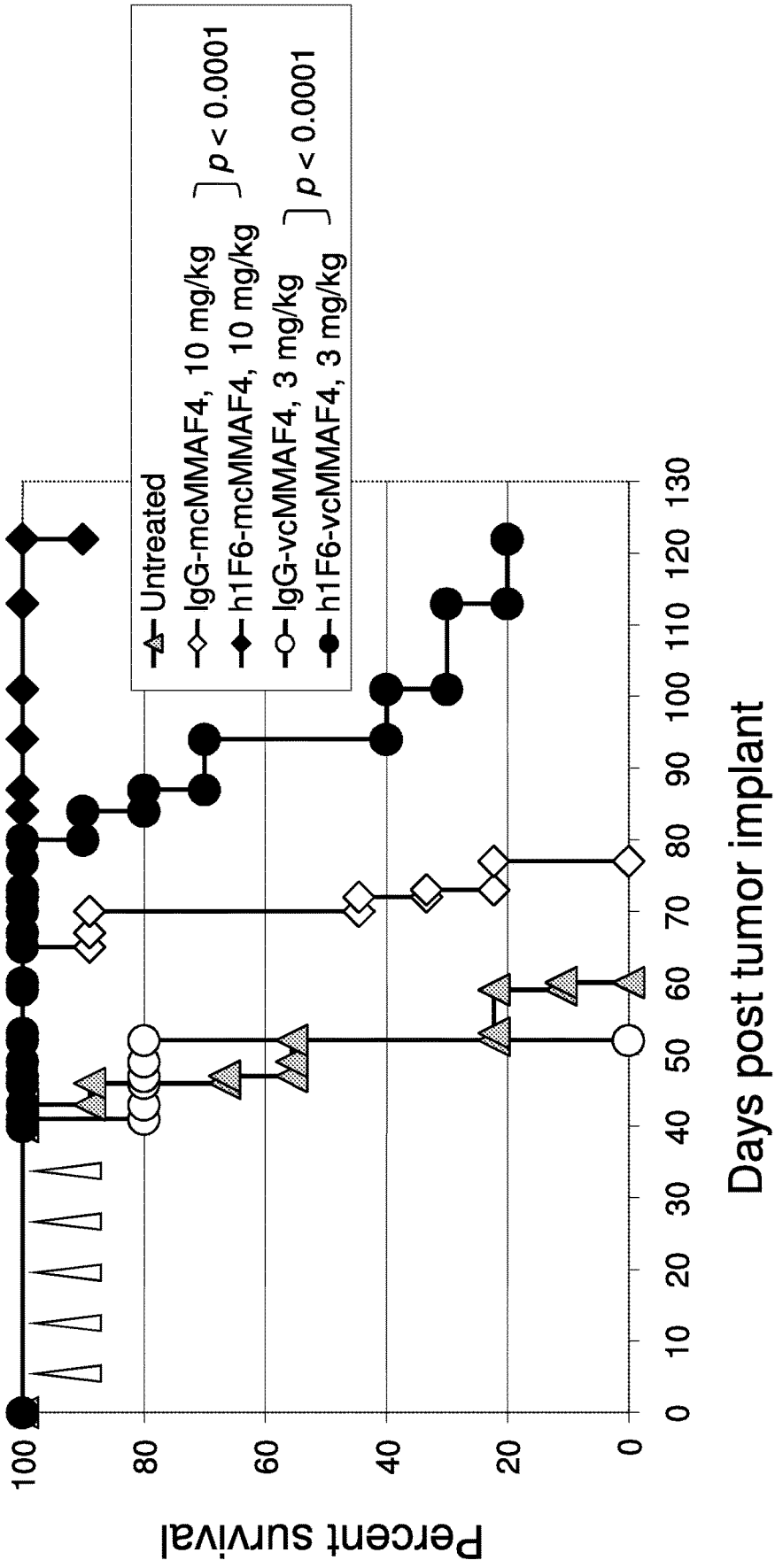

Percent with tumor size quadrupled
Days post implant
untreated
h1F6-mcMMAF4, 0.17 mg/kg, q4dx4*
h1F6-mcMMAF4, 0.17 mg/kg, q4dx10*
* p < 0.05
untreated
h1F6-vcMMAF4, 0.17 mg/kg, q4dx4*
h1F6-vcMMAF4, 0.17 mg/kg, q4dx10*
* p < 0.05
0
20
40
60
80
100
0
10
20
30
40
50

FIG. 11A


Untreated
IgG-mcMMAF4, 10 mg/kg
h1F6-mcMMAF4, 10 mg/kg
IgG-vcMMAF4, 3 mg/kg
h1F6-vcMMAF4, 3 mg/kg
p < 0.0001
p < 0.0001
Percent survival
100
80
60
40
20
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130
Days post tumor implant

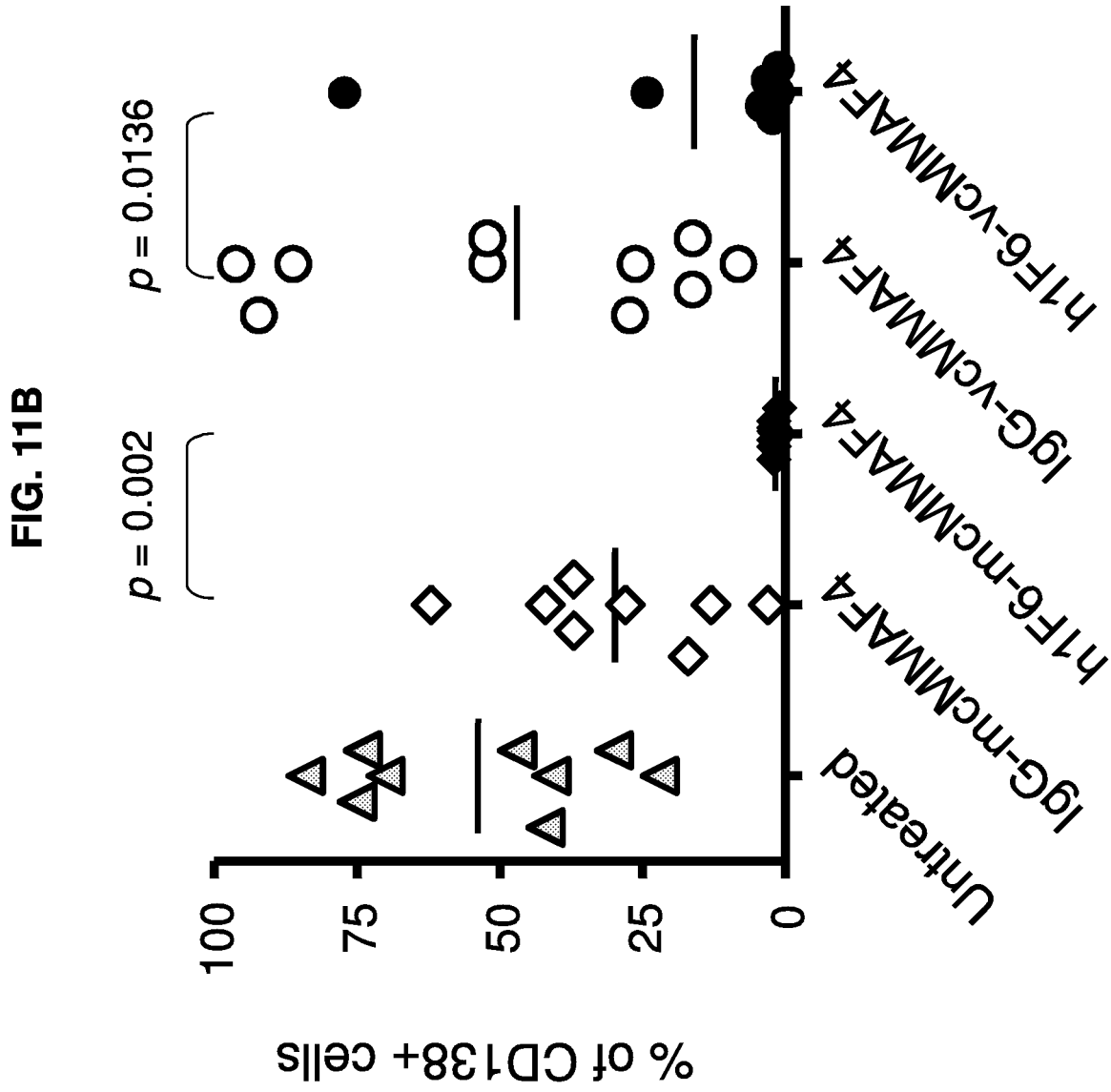

% of CD138+ cells
100
75
50
25
0
Untreated
IgG-mcMMAF-4
h1F6-mcMMAF-4
IgG-vcMMAF-4
h1F6-vcMMAF-4
p = 0.002
p = 0.0136

FIG. 11B

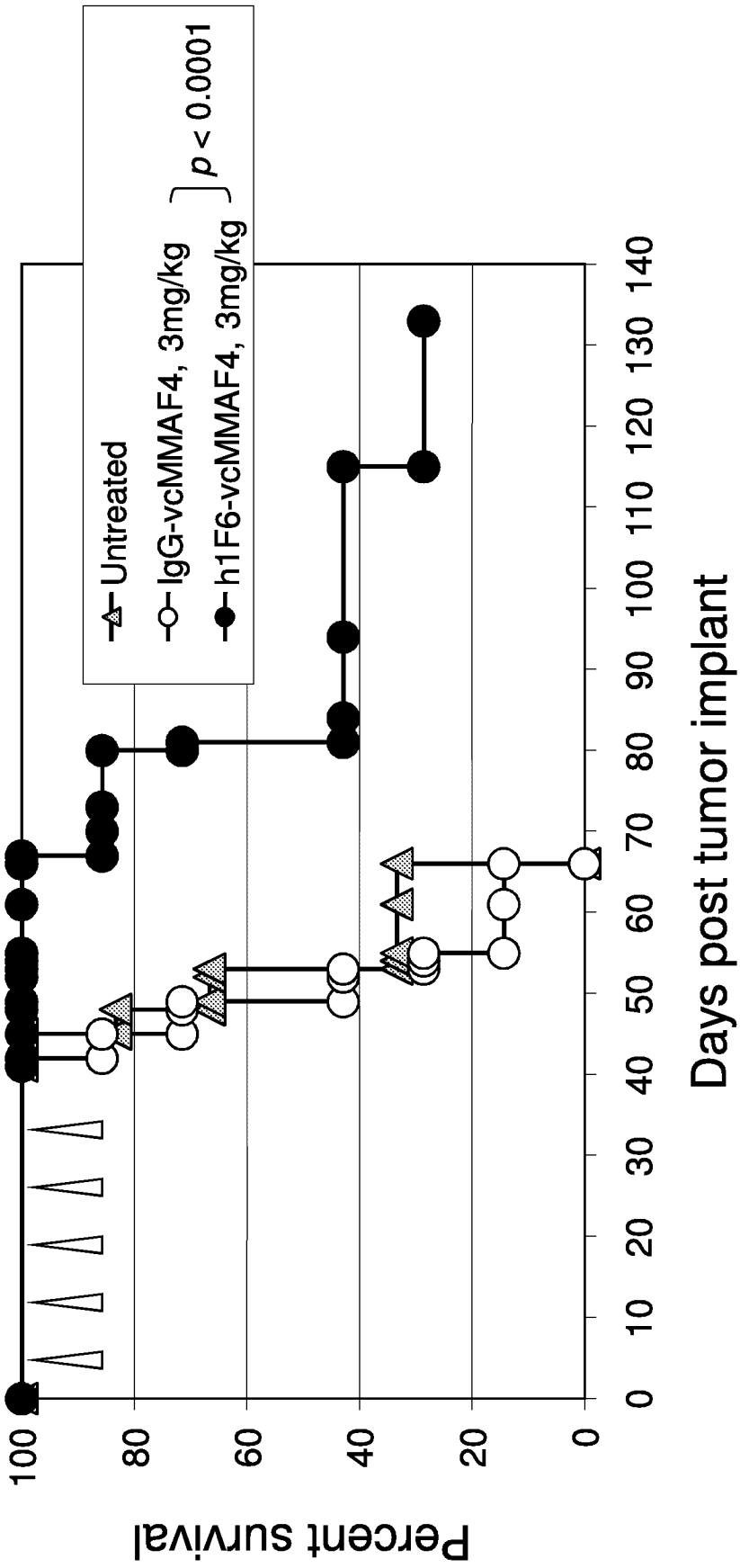

Untreated
IgG-vcMMAF4, 3mg/kg
h1F6-vcMMAF4, 3mg/kg
p < 0.0001
Percent survival
100
80
60
40
20
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140
Days post tumor implant

FIG. 12A

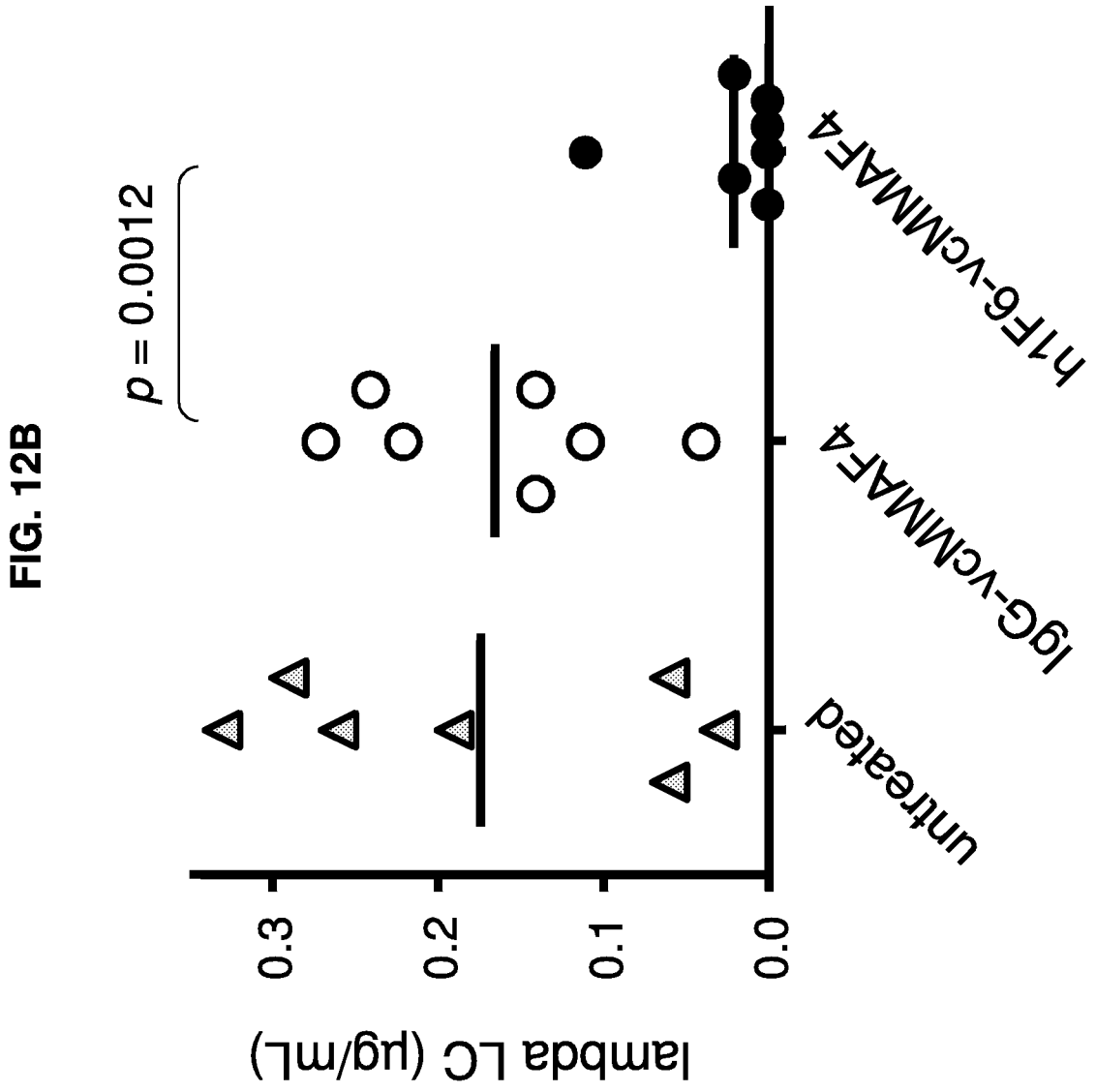

lambda LC (µg/mL)
0.0
0.1
0.2
0.3
untreated
IgG-vcMMAF4
h1F6-vcMMAF4
p = 0.0012

FIG. 12B

HUMANIZED ANTI-CD70 BINDING AGENTS AND USES THEREOF

RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 15/614,571 filed Jun. 5, 2017, which is a continuation of U.S. application Ser. No. 15/217,109 filed Jul. 22, 2016 now U.S. Pat. No. 9,701,752, which is a continuation of U.S. application Ser. No. 14/053,164, filed Oct. 14, 2013 now U.S. Pat. No. 9,428,585, which is a division of U.S. application Ser. No. 13/271,143, filed Oct. 11, 2011, now U.S. Pat. No. 8,562,987, which is a continuation of U.S. application Ser. No. 11/912,096, filed Oct. 23, 2008, now U.S. Pat. No. 8,067,546, which is a U.S. National Stage Application under 35 USC 371 of International Application No. PCT/US2006/015145, filed Apr. 19, 2006, which claims the benefit under 35 USC 119(e) of U.S. Provisional Patent Application No. 60/673,070, filed Apr. 19, 2005, each of which is incorporated by reference herein in its entirety.

REFERENCE TO A SEQUENCE LISTING

[0002] This application includes an electronic sequence listing in a file named "544705SEQLST.TXT", created on May 7, 2020 and containing 52,165 bytes, which is hereby incorporated by reference in its entirety for all purposes.

BACKGROUND

[0003] 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).

[0004] 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 medullar epithelial cells (Hintzen et al., 1994, supra; Hishima et al., 2000, *Am. J. Surg. Pathol.* 24:742-46).

[0005] 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.

[0006] Accordingly, there is a need for anti-CD70 antibodies and other CD70 binding agents 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. Such compounds would be useful therapeutic agents against cancers that express CD70 or immune disorders that are mediated by CD70-expressing cells. (The recitation of any reference in this application is not an admission that the reference is prior art to this application.)

BRIEF SUMMARY

[0007] The present invention provides CD70 antibodies and related CD70 binding agents and methods relating to the use of such binding agents for the prophylaxis or treatment of CD70-expressing cancers and immunological disorders where CD70-expressing cells are present. The CD70 binding agent, alone or in combination with a therapeutic agent, exerts a cytotoxic, cytostatic, and/or immunomodulatory effect on CD70-expressing cells.

[0008] In an aspect, CD70 binding agents are provided. The CD70 binding agent can be, for example, an antibody. In some embodiments, the binding agent includes at least one effector domain mediating at least an ADCC, ADCP or CDC response in the subject. In some embodiments, the binding agent exerts a cytostatic, cytotoxic or immunomodulatory effect in the absence of conjugation to a therapeutic agent. In some embodiments, the binding agent is conjugated to a therapeutic agent that exerts a cytotoxic, cytostatic or immunodulatory effect. The antibody can compete for binding to CD70 with monoclonal antibody 1F6 or 2F2.

[0009] In another aspect, a method of treating a CD70-expressing cancer in a subject is provided. The method generally includes administering to the subject an effective amount of a CD70 binding agent. In some embodiments, the binding agent includes at least one effector domain mediating at least an ADCC, ADCP or CDC response in the subject. In some embodiments, the binding agent exerts a cytostatic, cytotoxic or immunomodulatory effect in the absence of conjugation to a therapeutic agent. In some embodiments, the binding agent is conjugated to a therapeutic agent that exerts a cytotoxic, cytostatic or immunodulatory effect.

[0010] The CD70-binding agent can be, for example, an antibody. The antibody can include, for example, an effector domain of a human IgM or IgG antibody. The IgG antibody can be, for example, a human IgG1 or IgG3 subtype. In some embodiments, the antibody includes a human constant region. In some embodiments, the CD70 binding agent competes for binding to CD70 with monoclonal antibody

1F6 or 2F2. In other embodiments, the antibody is a humanized 1F6. In other embodiments, the antibody is a humanized 2F2. The antibody can be, for example, monovalent, divalent or multivalent.

[0011] The CD70-expressing cancer can be, a kidney tumor, a B cell lymphoma, a colon carcinoma, Hodgkin's Disease, multiple myeloma, Waldenström's macroglobulinemia, non-Hodgkin's lymphoma, a mantle cell lymphoma, chronic lymphocytic leukemia, acute lymphocytic leukemia, a nasopharyngeal carcinoma, brain tumor or a thymic carcinoma. The kidney tumor can be, for example, a renal cell carcinoma. The brain tumor can be, for example, a glioma, a glioblastoma, an astrocytoma or a meningioma. The subject can be, for example, a mammal, such as a human being.

[0012] In another aspect, a method for treating an immunological disorder is provided. The method includes administering to a subject an effective amount of a CD70 binding agent. In some embodiments, the binding agent includes at least one effector domain mediating at least an ADCC, ADCP or CDC response in the subject. In some embodiments, the binding agent exerts a cytostatic, cytotoxic or immunomodulatory effect in the absence of conjugation to a therapeutic agent. In some embodiments, the binding agent is conjugated to a therapeutic agent that exerts a cytotoxic, cytostatic or immunodulatory effect. The CD70 binding agent can be, for example, an antibody. The antibody can include, for example, an effector domain of a human IgM or IgG antibody. The IgG antibody can be, for example, a human IgG_1 or IgG_3 subtype. In some embodiments, the antibody includes a human constant region.

[0013] The immunological disorder can be, for example, a T cell-mediated immunological disorder. In some embodiments, the T cell mediated immunogical disorder comprises activated T cells expressing CD70. In some embodiments, resting T cells are not substantially depleted by administration of the antibody-drug conjugate. The T cell-mediated immunological disorder also can be, for example, rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosus (SLE), Type I diabetes, asthma, atopic dermatitus, allergic rhinitis, thrombocytopenic purpura, multiple sclerosis, psoriasis, Sjögren's syndrome, Hashimoto's thyroiditis, Graves' disease, primary biliary cirrhosis, Wegener's granulomatosis, tuberculosis, or graft versus host disease. In other embodiments, the immunological disorder is an activated B-lymphocyte disorder. The subject can be, for example, a mammal, such as a human being.

[0014] In a related aspect, also provided is a pharmaceutical composition for the treatment of a CD70-expressing cancer or an immunological disorder. The composition includes a CD70 binding agent and at least one pharmaceutically compatible ingredient. Further provided is a pharmaceutical kit including a container including a CD70 binding agent, wherein the agent is lyophilized, and a second container comprising a pharmaceutically acceptable diluent.

[0015] The present invention may be more fully understood by reference to the following detailed description of the invention, non-limiting examples of specific embodiments of the invention and the appended figures and sequence listing.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIG. **1** is an alignment of humanized 1F6 V_H humanized variants hV_HE (SEQ ID NO:6) and hV_HJ (SEQ ID NO:14) with 1F6 mV_H (residues 20-137 of SEQ ID NO:2) and human germline V_H exon V_H1-2 and J_H exon JH6 (collectively SEQ ID NO:31). In the alignment, a <•> indicates that the amino acid is identical to the murine residue. The highlighted lysine residue (K) at H46 of hV_HJ indicates a back mutation to the murine residue. Underlined amino acid residues indicate positions in CDR1 and CDR2 according to the Kabat definition, whereas boxed residues indicate positions in the corresponding CDR identified by the Chothia definition. A <^>, as at positions 37, 39, 45, 47, 95 and 97, indicates a residue in involved in the V_H/V_L interface.

[0017] FIG. **2** is an alignment of humanized 1F6 V_H humanized variants hV_HH (SEQ ID NO:10) and hV_HM (SEQ ID NO:18) with 1F6 mV_H (residues 20-137 of SEQ ID NO:2) and human germline V_H exon V_H1-18 and J_H exon JH6 (collectively SEQ ID NO:32). In the alignment, a <•> indicates that the amino acid is identical to the murine residue. The highlighted residues at H46, H67, H68, H69, H70, and H71 of hV_HM indicate back mutations to the murine residues. Similarly, the highlighted residues at H67, H68, H69, H70, H71, H80, H81, H82, and H82A of hV_HH indicate back mutations to the murine residues. Underlined amino acid residues indicate positions in CDR1, CDR2, and CDR3 according to the Kabat definition, whereas boxed residues indicate positions in the corresponding CDR identified by the Chothia definition. A <^>, as at positions 37, 39, 45, 47, 98, and 100 indicates a residue in involved in the V_H/V_L interface.

[0018] FIG. **3** is an alignment of humanized 1F6 V_L variant hV_LA (SEQ ID NO:24) with 1F6 mV_L (residues 21-132 of SEQ ID NO:22) and human germline V_κ exon B3 and J_κ exon Jκ-1 (collectively SEQ ID NO:33). In the alignment, a <•> indicates that the amino acid is identical to the murine residue. Underlined amino acid residues indicate positions in CDR1, CDR2, and CDR3 according to the Kabat definition, whereas boxed residues indicate positions in the corresponding CDR identified by the Chothia definition. A <^>, as at positions 42, 44, 50, 52, and 93, indicates a residue in involved in the V_H/V_L interface.

[0019] FIGS. **4**A-B show that humanized 1F6 anti-CD70 antibodies mediate antibody-dependent cellular cytotoxicity (ADCC). $Na_2{}^{51}CrO_4$-labeled target cells (WIL2-S B lymphoblastoid cells, 786-O renal cell carcinoma cells, and 769-P renal cell carcinoma cells) were coated with antibody and incubated with peripheral blood mononuclear cells (PBMC) at an effector to target ratio of 10 $CD16^+$ (FcγIII receptor) cells to 1 target cell. After 4 hours, the supernatants from lysed cells were measured on a scintillation counter. The percent specific lysis was calculated as {(test sample cpm−spontaneous cpm)÷(total cpm−spontaneous cpm)}× 100. Points represent the mean±standard deviation of triplicate samples. FIG. **4**A shows the ADCC activity mediated by humanized 1F6 variants HHLA, HJLA and HELA compared to non-binding antibody control $hIgG_1$ and murine 1F6 antibody. FIG. **4**B shows antibody-directed lysis of renal cell carcinoma cell lines mediated by chimeric 1F6 and humanized 1F6 variant HJLA, and $hIgG_1$.

[0020] FIG. **5** shows that humanized 1F6 anti-CD70 variant HJLA mediates complement-dependent cellular cytotoxicity (CDC). LP-1, MHH PreB-1 and WIL-S target cells were mixed with chimeric 1F6, humanized 1F6 (HJLA) or non-binding human Ig in the presence of human serum as a source of complement. After 2 hours at 37° C., propidium

iodide was added to determine cell viability as measured by flow cytometry, and the amount of lytic activity was calculated. Bars represent the mean±standard deviation of triplicate samples.

[0021] FIG. **6** shows that humanized 1F6 anti-CD70 antibodies mediate antibody-dependent cellular phagocytosis (ADCP). 786-O $CD70^+$ renal cell carcinoma target cells were labeled with a red fluorescent cell membrane dye (PKH26, Sigma-Aldrich, Inc., St. Louis, Mo.) and then coated with chimeric 1F6, humanized 1F6 (HJLA) or non-binding human Ig for 30 minutes on ice. Labeled, antibody-treated target cells were mixed with monocyte-derived macrophages at a ratio of 1 macrophage to 4 target cells for 1 hour at 37° C. Macrophages were stained with an Alexa Fluor® 488 (Molecular Probes, Inc., Eugene, Oreg.) anti-CD11b antibody and the percent phagocytic activity was determined by the percentage of macrophages exhibiting dual fluorescence when analyzed by flow cytometry.

[0022] FIGS. **7**A-C shows humanized 1F6 anti-CD70 antibody prolongs survival of mice in xenograft models of disseminated lymphoma and multiple myeloma. (FIG. **7**A) Survival of mice injected with Raji cells and treated with humanized 1F6 antibody or control non-binding antibody. Treatment was initiated one day after tumor cell injection and was administered by intraperitoneal injection every four days for a total of six doses (n=10 per group). (FIGS. **7**B-C, left panels) Survival of mice injected with L363- or MM.1S-cells and treated with humanized 1F6 starting one day after cell implant. The antibody was administered by intravenous injection once weekly for a total of five doses. Mice were monitored twice weekly and were euthanized upon manifestation of disease (n=7 per group). (FIGS. **7**B-C, right panels) Analyis of λ light chain concentrations in sera collected from mice injected with L363- or MM.1S-cells. Samples were collected on days 35 and 42 post tumor injection, respectively. In all studies, p values given are between humanized 1F6-treated groups and the untreated group.

[0023] FIGS. **8**A-B shows humanized 1F6 mediates depletion of antigen-specific CD8+/Vβ17+ cells. PBMCs from a normal HLA-A 0201 donor were stimulated with the M1 peptide. (FIG. **8**A) Peptide-stimulated cultures were untreated or treated with concurrent addition of graded doses of humanized 1F6 antibody, as indicated. The percent CD8+/Vβ17+ cells after 9 days was determined by flow cytometry. (FIG. **8**B) Peptide-stimulated cultures were untreated or treated on day 0 with 1 μg/ml humanized 1F6 in the absence (solid bars) or presence (hatched bars) of 10 μg/ml antibody specific for FcγRIII (CD16). The percent CD8+/Vβ17+ cells after 9 days was determined by flow cytometry.

[0024] FIG. **9** shows minimal impact of anti-CD70 1F6 antibody on bystander resting T cells. PBMCs from a normal HLA-A 0201 donor were untreated (no stim) or stimulated with M1 peptide (peptide stim) in the presence or absence of 1 μg/mL c1F6. After nine days in culture, Vβ TCR representation among CD4 and CD8 cells from each group was analyzed by flow cytometry using the IOTest® Beta Mark TCR Vβ Repertoire Kit.

[0025] FIGS. **10**A-B shows a mouse Xenograft model of Renal Cell Carcinoma. (FIG. **10**A) Subcutaneous 786-O tumors were initiated in nude mice by implanting tumor fragments (N=5 or 6/group) of approximately 30 mm^3. Tumor growth was allowed to establish and treatment began when average tumor size within each group was approximately 100 mm^3. h1F6-mcMMAF4 or h1F6-vcMMAF4 at the indicated doses was administered at a q4d×4 schedule beginning on day 17 after tumor implantation, as indicated by the arrows. Cross-strikes indicate when animals with tumors >1000 mm^3 were euthanized. (FIG. **10**B) 786-O tumor implantation and treatment initiation are the same as given in (FIG. **10**A). Groups of mice (N=5-7) were administered with h1F6-mcMMAF4 or h1F6-vcMMAF4 at 0.17 mg/kg at a q4d×4 or q4d×10 schedule beginning on day 13 after tumor implantation. Tumor growth is represented by Kaplan-Meier plots. An event was registered when a mouse with a tumor quadrupled in size compared to day 13 when treatment began. Mice with tumors that did not quadruple in size at the end of the experiment on day 43 were censored. The log-rank test was used to generate p values between treatment groups and the untreated group.

[0026] FIGS. **11**A-B shows a mouse Xenograft Model of Multiple Myeloma. (FIG. **11**A) Ten million MM-1S cells were injected intravenously into each SCID mouse. Groups of mice (N=8-10) were left untreated, received IgG-vcMMAF4, IgG-mcMMAF4, h1F6-vcMMAF4, or h1F6-mcMMAF4 at the specified doses on a q7d×5 schedule, as indicated by the arrows. Mice showing symptoms of hind limb paralysis, hunched posture, cranial swelling, and/or scruffy coat were euthanized, and the percent survival of each group was plotted. The log-rank test was used to generate p values between treatment groups and the control groups. (FIG. **11**B) Bone marrow cells were recovered from the femurs of euthanized mice due to the above disease symptoms or on day 122 post tumor cell implantation when the experiment was terminated. The percentage of CD138-expressing MM-1S cells in the femers of each mouse was determined by flow cytometry. The Mann-Whitney test was used to derived p values between the indicated groups.

[0027] FIGS. **12**A-B shows a mouse Xenograft model of Multiple Myeloma. (FIG. **12**A) Ten million L363 cells were injected intravenously into each SCID mouse. Groups of mice (N=7) were left untreated, received IgG-vcMMAF4, or h1F6-vcMMAF4 at the specified doses on a q7d×5 schedule as indicated by the arrows. Mice showing palpable tumor masses were euthanized, and the percent survival of each group was plotted. The log-rank test was used to generate the p value between the treated group and the untreated group. (FIG. **12**B) Serum samples were obtained from mice 40 days after tumor implant. The concentration of human λ light chain in the serum of each mouse was determined by ELISA. The Mann-Whitney test was used to derive p values between the indicated groups.

DETAILED DESCRIPTION

[0028] The present invention provides CD70 binding agents and methods for using such binding agents for the prophylaxis or treatment of CD70-expressing cancers and immunological disorders. The CD70 binding agent specifically binds to CD70 (e.g., the extracellular domain). The binding agent may include at least one effector domain mediating an ADCC, ADCP and/or CDC response. The binding agent may exert a cytostatic, cytotoxic or immunomodulatory effect in the absence of conjugation to a therapeutic agent. The binding agent may be conjugated to a therapeutic agent that exerts a cytotoxic, cytostatic or immunodulatory effect.

[0029] In one aspect, the compositions and methods relate to CD70 binding agents, such as antibodies and antibody

derivatives. The anti-CD70 antibody can be a monoclonal, chimeric or humanized antibody, or a fragment or derivative thereof. In some embodiments, the anti-CD70 antibody includes an antibody constant region or domain. The antibody constant region or domain can be, for example, of the IgG subtype. In an exemplary embodiment, the anti-CD70 antibody, fragment or derivatives thereof, competes with the murine monoclonal antibody (mAb) 1F6 or 2F2 for binding to CD70 and comprises human antibody constant region sequences. In another exemplary embodiment, the anti-CD70 antibody, or fragment or derivative thereof, has an effector domain (e.g., an Fc portion) that can interact with effector cells or complement to mediate a cytotoxic, cytostatic, and/or immunomodulatory effect that results in the depletion or inhibition of the proliferation of CD70-expressing cells. In another exemplary embodiment, the anti-CD70 antibody lacks effector function. In another exemplary embodiment, the anti-CD70 antibody is conjugated to a therapeutic agent.

[0030] Also included are kits and articles of manufacture comprising a CD70 binding agent (e.g., a humanized anti-CD70 antibody).

[0031] For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections which follow.

I. Definitions and Abbreviations

[0032] 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.

[0033] 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.

[0034] 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).

[0035] As used herein, the term "functional" in the context of a CD70 binding agent indicates that the binding agent is capable of binding to CD70.

[0036] The terms "inhibit" or "inhibition of" as used herein means to reduce by a measurable amount, or to prevent entirely.

[0037] 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.

[0038] "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).

[0039] 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 and 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.

[0040] 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.

[0041] 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), comple-

ment dependent cytotoxicity (CDC) and/or antibody dependent cellular phagocytosis (ADCP).

[0042] 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, IgA, 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.

[0043] The terms "antibody", "anti-CD70 antibody", "humanized anti-CD70 antibody", and "variant humanized anti-CD70 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.

[0044] 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 Kohler et al., 1975, *Nature* 256: 495, or recombinant DNA methods known in the art (see, e.g., U.S. Pat. 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.

[0045] 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.

[0046] 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. Pat. 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. Pat. Nos. 5,807,715; 4,816,567; and 4,816,397.)

[0047] The terms "antibody fragment", "anti-CD70 antibody fragment", "humanized anti-CD70 antibody fragment", and "variant humanized anti-CD70 antibody fragment" refer to a portion of a full-length anti-CD70 antibody in which a variable region or a functional capability is retained, for example, specific CD70 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.)

[0048] 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, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315).

[0049] 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.

[0050] 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.

[0051] 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.

[0052] 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.

[0053] 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.

[0054] The humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG_1, IgG_2, IgG_3 and IgG_4. 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., IgG_1). Where such cytotoxic activity is not desirable, the constant domain may be of another class (e.g., IgG_2). 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.

[0055] 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%.

[0056] 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,* 4^{th} 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,* 6^{th} ed., Janeway et al., Garland Science, N. Y., 2005, Chapter 2).

[0057] 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.

[0058] 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.

[0059] 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.

[0060] "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.

[0061] "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.

[0062] 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.

[0063] 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 I^{131}, I^{125}, Y^{90}, and Re^{186}), 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, 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.

[0064] A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such a thiotepa and cyclosphosphamide (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 Oct. 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, chlomaphazine, 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 calichemicin gamma1I and calicheamicin phi1I, 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 chromomophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (Adriamycin™) (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, and deoxydoxorubicin), epirubucin, 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 a 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, epitiostanol, mepitiostane, testolactone; anti-adranals 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, N.J.) 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.

[0065] 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.

[0066] 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.

[0067] The term "immunomodulatory effect" as used herein refers to a stimulation (immunostimulatory) or inhibition (immunosuppressive) of the development or mainte-

nance 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.

[0068] 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.

[0069] 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.

[0070] 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.

[0071] 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.

[0072] 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.

[0073] 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.

[0074] 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.

[0075] 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.

[0076] 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).

[0077] 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)×100). In some embodiments, the two sequences are the same length.

[0078] 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).

[0079] 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.

[0080] 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).

[0081] 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.

[0082] 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.

[0083] 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.

[0084] 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.

[0085] 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 or disorders to be treated herein include cancer, hematological malignancies, benign and malignant tumors, leukemias and lymphoid malignancies, carcinomas, and inflammatory, angiogenic and immunologic disorders. Specific examples of disorders are disclosed infra.

[0086] 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.

[0087] As used herein, the terms "prevention" or "prevent" refer to administration of an anti-CD70 binding agent to a subject before the onset of a clinical or diagnostic symptom of a CD70-expressing cancer or immunological disorder (e.g., administration to an individual with a predisposition or at a high risk of acquiring the CD70-expressing cancer or immunological disorder) to (a) block the occurrence or onset of the CD70-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 cancer or immunological disorder, or (c) to lessen the likelihood of the onset of the CD70-expressing cancer or immunological disorder.

[0088] 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.

[0089] 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.

[0090] 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.

[0091] 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.

[0092] 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.

[0093] 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.

[0094] 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.

[0095] The term "effective amount" refers to the amount of an anti-CD70 binding agent (e.g., an antibody or derivative or other binding agent) that is sufficient to inhibit the occurrence or ameliorate one or more clinical or diagnostic symptoms of a CD70-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 cancer or immunological disorder.

[0096] 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, efficacy can be measured by assessing the time to disease progression (TTP), or determining the response rates (RR).

[0097] 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 is administered.

[0098] 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. The anti-CD70 binding agent 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.

[0099] "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. 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.

[0100] The abbreviation "AFP" refers to dimethylvaline-valine-dolaisoleuine-dolaproine-phenylalanine-p-phenylenediamine.

[0101] The abbreviation "MMAE" refers to monomethyl auristatin E.

[0102] The abbreviation "AEB" refers to an ester produced by reacting auristatin E with paraacetyl benzoic acid.

[0103] The abbreviation "AEVB" refers to an ester produced by reacting auristatin E with benzoylvaleric acid.

[0104] The abbreviation "MMAF" refers to dovaline-valine-dolaisoleunine-dolaproine-phenylalanine.

[0105] The abbreviations "fk" and "phe-lys" refer to the linker phenylalanine-lysine.

II. Anti-CD70 Antibodies and Derivatives Thereof

[0106] The compositions and methods described herein encompass the use of a CD70 binding agent that specifically binds to CD70. The CD70 binding agent may exert a cytotoxic, cytostatic or immunomodulatory effect on CD70-expressing cancer cells, activated immune cells or other target cells. The CD70 binding agent can be, for example, an anti-CD70 antibody, an antigen-binding fragment of an anti-CD70 antibody, a derivative thereof, or other CD70-binding agent comprising at least one complementarity determining region (CDR) of a CD70-binding antibody.

[0107] In one aspect, the CD70 binding agent comprises one or more complementarity determining regions (CDRs) identical, substantially identical or substantially similar to one or more CDR(s) of monoclonal antibody 1F6. (The nucleic acid and amino acid sequences of the heavy and light chain variable regions of 1F6 are set forth in SEQ ID NO:1 and SEQ ID NO:2, and SEQ ID NO: 21 and SEQ ID NO: 22, respectively, and are disclosed in International Patent Publication No. WO 04/073656; the disclosure of which is incorporated by reference herein.) For example, the binding agent can include a heavy chain CDR and/or a light chain CDR that is identical or substantially identical or substantially similar to a corresponding heavy chain CDR (H1, H2, or H3 regions) or corresponding light chain CDR (L1, L2, or L3 regions) of mAb 1F6. In typical embodiments, the anti-CD70 binding agent has two or three heavy chain CDRs and/or two or three light chain CDRs that are identical, substantially identical or substantially similar to corresponding heavy and/or light chain CDRs of mAb 1F6.

[0108] For example, in some embodiments, where the anti-CD70 binding agent has at least one heavy chain CDR substantially identical or substantially similar to a heavy chain CDR of mAb 1F6, the binding agent can further include at least one light chain CDR that is substantially identical or substantially similar to a light chain CDR of mAb 1F6.

[0109] In some embodiments, the anti-CD70 binding agent includes a heavy or light chain variable domain, the variable domain having (a) a set of three CDRs identical, substantially identical or substantially similar to corresponding CDRs of mAb 1F6, and (b) a set of four variable region framework regions from a human immunoglobulin. For example, an anti-CD70 antibody can include a heavy and/or light chain variable domain(s), the variable domain(s) having (a) a set of three CDRs, in which the set of CDRs are from monoclonal antibody 1F6, and (b) a set of four framework regions derived from a human IgG. The antibody can optionally include a hinge region. In an exemplary embodiment, the anti-CD70 antibody is a fully humanized antibody.

[0110] In another aspect, the CD70 binding agent comprises one or more complementarity determining regions (CDRs) substantially identical or substantially similar to one or more CDR(s) of monoclonal antibody 2F2. (The nucleic acid and amino acid sequences of the heavy and light chain variable regions of 2F2 are set forth in SEQ ID NO:27 and SEQ ID NO:28, and SEQ ID NO: 29 and SEQ ID NO: 30, respectively, and are disclosed in International Patent Publication No. WO 04/073656; the disclosure of which is incorporated by reference herein.) For example, the binding agent can include a heavy chain CDR and/or a light chain CDR that is identical or substantially identical or substantially similar to a corresponding heavy chain CDR (H1, H2, or H3 regions) or corresponding light chain CDR (L1, L2, or L3 regions) of mAb 2F2. In typical embodiments, the anti-CD70 binding agent has two or three heavy chain CDRs and/or two or three light chain CDRs that are identical, substantially identical or substantially similar to corresponding heavy and/or light chain CDRs of mAb 2F2.

[0111] For example, in some embodiments, where an anti-CD70 antibody has at least one heavy chain CDR substantially identical or substantially similar to a heavy chain CDR of mAb 2F2, the antibody or derivative thereof can further include at least one light chain CDR that is substantially identical or substantially similar to a light chain CDR of mAb 2F2.

[0112] In some embodiments, the anti-CD70 binding agent includes a heavy or light chain variable domain, the variable domain having (a) a set of three CDRs identical, substantially identical or substantially similar to corresponding CDRs of mAb 2F2, and (b) a set of four variable region framework regions from a human immunoglobulin. For example, an anti-CD70 antibody can include a heavy and/or light chain variable domain(s), the variable domain(s) hav-

ing (a) a set of three CDRs, in which the set of CDRs are from monoclonal antibody 2F2, and (b) a set of four framework regions derived from a human IgG. The antibody can optionally include a hinge region. In an exemplary embodiment, the anti-CD70 antibody is a fully humanized antibody.

[0113] In some embodiments, the framework regions are chosen from human germline exon V_H, J_H, V_κ and J_κ sequences. For example, acceptor sequences for humanization of FR of a c1F6 V_H domain can be chosen from germline V_H exons V_H1-18 (Matsuda et al., 1993, *Nature Genetics* 3:88-94) or V_H1-2 (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). In other examples, germline V_κ exon B3 (Cox et al., 1994, *Eur. J. Immunol.* 24:827-836) and J_κ exon Jκ-1 (Hieter et al., 1982, *J. Biol. Chem.* 257:1516-1522) can be chosen as acceptor sequences for c1F6 V_L domain humanization.

[0114] In some embodiments, the sequence of the framework region of the humanized anti-CD70 antibody includes a derivative of the acceptor human germline exon used, including derivatives in which mouse donor residues are reintroduced. These residues include reintroduction of the mouse donor residue at one or more of positions H46, H67, H68, H69, H70, H71, H80, H81, H82, H82A and H91 in the V_H domain, according to the Kabat numbering convention.

[0115] The following table indicates the regions of humanized 1F6 to which each SEQ ID NO. corresponds.

TABLE 1

MOLECULE	NUCLEOTIDE OR AMINO ACID	SEQ ID NO
c1F6 Heavy Chain Variable Region	Nucleotide	1
c1F6 Heavy Chain Variable Region	Amino Acid	2
h1F6 hV_H-D + $hIgG_1$ Constant Domain	Nucleotide	3
h1F6 hV_H-D + $hIgG_1$ Constant Domain	Amino Acid	4
h1F6 hV_H-E	Nucleotide	5
h1F6 hV_H-E	Amino Acid	6
h1F6 hV_H-E + $hIgG_1$ Constant Domain	Nucleotide	7
h1F6 hV_H-E + $hIgG_1$ Constant Domain	Amino Acid	8
h1F6 hV_H-H	Nucleotide	9
h1F6 hV_H-H	Amino Acid	10
h1F6 hV_H-H + $hIgG_1$ Constant Domain	Nucleotide	11
h1F6 hV_H-H + $hIgG_1$ Constant Domain	Amino Acid	12
h1F6 hV_H-J	Nucleotide	13
h1F6 hV_H-J	Amino Acid	14
h1F6 hV_H-J + $hIgG_1$ Constant Domain	Nucleotide	15
h1F6 hV_H-J + $hIgG_1$ Constant Domain	Amino Acid	16
h1F6 hV_H-M	Nucleotide	17
h1F6 hV_H-M	Amino Acid	18
h1F6 hV_H-M + $hIgG_1$ Constant Domain	Nucleotide	19
h1F6 hV_H-M + $hIgG_1$ Constant Domain	Amino Acid	20
c1F6 Light Chain Variable Region	Nucleotide	21
c1F6 Light Chain Variable Region	Amino Acid	22
hV_LA	Nucleotide	23
hV_LA	Amino Acid	24
hV_LA + human κ constant domain	Nucleotide	25
hV_LA + human κ constant domain	Amino Acid	26
c2F2 Heavy Chain Variable Region	Nucleotide	27
c2F2 Heavy Chain Variable Region	Amino Acid	28
c2F2 Light Chain Variable Region	Nucleotide	29
c2F2 Light Chain Variable Region	Amino Acid	30

[0116] In some embodiments, the CD70 binding agent can be a humanized antibody or antigen-binding fragment of antibody 1F6 or 2F2. In some embodiments, the antibody or antigen-binding fragment comprises a polypeptide chain having the amino acid sequence of SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:18, or amino acids 20-137 of SEQ ID NO:4. In some embodiments, the antibody or antigen-binding fragment comprises a polypeptide chain having the amino acid sequence of SEQ ID NO:24.

[0117] In some embodiments, the antibody or antigen-binding fragment comprises a polypeptide chain that is at least 80% identical to the amino acid sequence of SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:18, or amino acids 20-137 of SEQ ID NO:4. In some embodiments, the antibody or antigen-binding fragment comprises a polypeptide chain that is at least 85% identical to the amino acid sequence of SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:18, or amino acids 20-137 of SEQ ID NO:4. In some embodiments, the antibody or antigen-binding fragment comprises a polypeptide chain that is at least 90% identical to the amino acid sequence of SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:18, or amino acids 20-137 of SEQ ID NO:4. In some embodiments, the antibody or antigen-binding fragment comprises a polypeptide chain that is at least 95% identical to the amino acid sequence of SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:18, or amino acids 20-137 of SEQ ID NO:4. In some embodiments, the antibody or antigen-binding fragment comprises a polypeptide chain that is at least 99% identical to the amino acid sequence of SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:18, or amino acids 20-137 of SEQ ID NO:4. In some embodiments, the polypeptide does not have the amino acid sequence of the heavy chain variable region of antibody 1F6 or 2F2.

[0118] In some embodiments, the antibody or antigen-binding fragment comprises a polypeptide chain that is at least 80% identical to the amino acid sequence of SEQ ID NO:24. In some embodiments, the antibody or antigen-binding fragment comprises a polypeptide chain that is at least 85% identical to the amino acid sequence of SEQ ID NO:24. In some embodiments, the antibody or antigen-binding fragment comprises a polypeptide chain that is at least 90% identical to the amino acid sequence of SEQ ID NO:24. In some embodiments, the antibody or antigen-binding fragment comprises a polypeptide chain that is at least 95% identical to the amino acid sequence of SEQ ID NO:24. In some embodiments, the antibody or antigen-binding fragment comprises a polypeptide chain that is at least 99% identical to the amino acid sequence of SEQ ID NO:24. In some embodiments, the polypeptide does not have the amino acid sequence of the light chain variable region of antibody 1F6 or 2F2.

[0119] In some embodiments, the anti-CD70 binding agent competes with monoclonal antibody 1F6 or 2F2 for binding to human CD70. In some embodiments, the CD70 binding agent does not induce an agonistic or antagonistic signal when binding to CD70 (e.g., does not stimulate proliferation). In some embodiments, the CD70 binding agent blocks binding of CD27 to CD70 by at least 20%, at least 30%, at least 40%, at least 50%, at least 60, at least 70%, at least 80% or at least 90%.

[0120] 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 lym-

phocytes 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).

[0121] 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.

[0122] 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.

[0123] The CD70-binding agent optionally can be conjugated to a therapeutic agent, such as a cytotoxic, cytostatic or immunomodulatory agent. Suitable therapeutic agents are described herein.

[0124] 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 a 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).

[0125] 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. Pat. Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; and U.S. Pat. No. 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 antibodies 2F2 and 1F6) and a second cell surface receptor or receptor complex that mediates ADCC, ADCP, and/or CDC, such as CD16/FcγRIII, CD64/FcγRI, 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.

[0126] Anti-CD70 antibodies and derivatives thereof and other binding agents may also be described or specified in terms of their binding affinity to CD70. Typical binding affinities include those with a dissociation constant or Kd 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, $5\times^{-13}$M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0127] 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, N.Y., 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. Pat. 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).

[0128] Examples of techniques that can be used to produce single-chain antibodies include those described in U.S. Pat. Nos. 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.

[0129] 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.

[0130] 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.

[0131] 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).

[0132] 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.

[0133] 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.

[0134] 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. Pat. 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. Pat. 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. Pat. No. 5,565,332) (all of these references are incorporated by reference herein).

[0135] 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. Pat. 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. Pat. 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.

[0136] 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.

[0137] 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.

[0138] 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.

[0139] 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.

[0140] 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. Pat. Nos. 5,155,027; 5,336,603; 5,359,046; and 5,349,053; EP 0 367 166; and WO 96/04388.)

[0141] 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.

[0142] 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. Pat. 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 antibodies 2F2 or 1F6) and an antibody that binds to a surface receptor or receptor complex that mediates ADCC, phagocytosis, and/or CDC, such as CD16/FcgRIII, CD64/FcgRI, 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.

[0143] In some embodiments, the anti-CD70 antibody or derivative thereof competitively inhibits binding of mAb 1F6 or 2F2 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 or 2F2 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 or 2F2 to CD70 by at least 80%, at least 85%, at least 90%, or at least 95%.

[0144] 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.)

[0145] 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., ^{3}H 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 or 2F2) can also be determined using radioimmunoassays. In this case, CD70 is incubated with the antibody of interest conjugated to a labeled compound (e.g., ^{3}H 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.

[0146] 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.

[0147] 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. Pat. 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.

[0148] 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).

[0149] 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).

[0150] 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.

[0151] 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.

[0152] 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).

[0153] 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.

[0154] 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, Calif., 91311), and the "HA" tag, which corre-

sponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., 1984, *Cell* 37:767), and the "flag" tag.

[0155] 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.

[0156] 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.

[0157] 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. Other CD70-Binding Agents

[0158] Further CD70-binding agents include fusion proteins (i.e., proteins that are recombinantly fused or chemically conjugated, including both covalent and non-covalent conjugation) to heterologous proteins (of typically at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or at least 100 amino acids). Such CD70-binding agents can include a portion that binds to CD70 and an immunoglobulin effector domain or a functional equivalent thereof. As used herein, a functional equivalent of immunoglobulin effector domain binds 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. The fusion protein does not necessarily need to be direct, but may occur through linker sequences.

[0159] For example, a CD70-binding agent can be produced recombinantly by fusing the coding region of one or more of the CDRs or the variable region of an anti-CD70 antibody in frame with a sequence coding for a heterologous protein. The heterologous protein can include, for example, an effector domain, a functional equivalent thereof or other functional domain to provide one or more of the following characteristics: promote stable expression; provide a means of facilitating high yield recombinant expression; provide a cytostatic, cytotoxic or immunomodulatory activity; and/or provide a multimerization domain.

[0160] In some embodiments, the CD70-binding agent can include one or more CDRs from an antibody that binds to CD70 and depletes or inhibits the proliferation of CD70-expressing cells alone, without conjugation to a cytotoxic agent.

IV. Methods to Improve Effector Functions of Anti-CD70-Targeting Agents

[0161] In some embodiments, the effector function of a CD70-binding agent can be augmented by improving its effector functions using one or more antibody engineering approaches known in the art. Illustrative, non-limiting examples for such approaches are provided below.

[0162] ADCC and ADCP are mediated through the interaction of cell-bound antibodies with Fcγ receptors (FcγR) expressed on effector cells. Both the glycosylation status and primary amino acid sequence of the IgG Fc region have functional effects on the Fcγ-FcγR interaction. A stronger Fcγ-FcγR interaction is associated with better target cell killing by effector cells.

[0163] Oligosaccharides covalently attached to the conserved Asn297 are involved in the Fc region of an IgG to bind FcγR (Lund et al., 1996, *J. Immunol.* 157:4963-69; Wright and Morrison, 1997, *Trends Biotechnol.* 15:26-31). Engineering of this glycoform on IgG can significantly improve IgG-mediated ADCC. Addition of bisecting N-acetylglucosamine modifications (Umana et al., 1999, *Nat. Biotechnol.* 17:176-180; Davies et al., 2001, *Biotech. Bioeng.* 74:288-94) to this glycoform or removal of fucose (Shields et al., 2002, *I Biol. Chem.* 277:26733-40; Shinkawa et al., 2003, *J. Biol. Chem.* 278:6591-604; Niwa et al., 2004, *Cancer Res.* 64:2127-33) from this glycoform are two examples of IgG Fc engineering that improves the binding between IgG Fc and FcγR, thereby enhancing Ig-mediated ADCC activity.

[0164] A systemic substitution of solvent-exposed amino acids of human IgG1 Fc region has generated IgG variants with altered FcγR binding affinities (Shields et al., 2001, *J. Biol. Chem.* 276:6591-604). When compared to parental IgG1, a subset of these variants involving substitutions at Thr256/Ser298, Ser298/Glu333, Ser298/Lys334, or Ser298/Glu333/Lys334 to Ala demonstrate increased in both binding affinity toward FcγR and ADCC activity (Shields et al., 2001, *J. Biol. Chem.* 276:6591-604; Okazaki et al., 2004, *J. Mol. Biol.* 336:1239-49).

[0165] Antibody-mediated CDC begins with the binding of C1q to cell bound IgG molecules. Specific amino acid residues on human IgG1 responsible for C1q binding and species-specific differences of C1q binding have been reported (Idusogie et al., 2000, *J. Immunol.* 164:4178-4184). Complement fixation activity of antibodies have been improved by substitutions at Lys326 and Glu333; for example, such substitutions can improve both C1q binding and CDC activity of the human IgG1 antibody rituximab (Idusogie et al., 2001, *J. Immunol.* 166:2571-2575). The same substitutions on a human IgG2 backbone can convert an antibody isotype that binds poorly to C1q and is severely deficient in complement activation activity to one that can both bind C1q and mediate CDC (Idusogie et al., 2001, *J. Immunol.* 166:2571-75). Several other methods have also been applied to improve complement fixation activity of antibodies. For example, the grafting of an 18-amino acid carboxyl-terminal tail piece of IgM to the carboxyl-termini of IgG greatly enhances their CDC activity. This is observed even with IgG4, which normally has no detectable CDC activity (Smith et al., 1995, *J. Immunol.* 154:2226-36). Also, substituting Ser444 located close to the carboxy-terminal of IgG1 heavy chain with Cys induced tail-to-tail dimerization of IgG1 with a 200-fold increase of CDC activity over monomeric IgG1 (Shopes et al., 1992, *J. Immunol.* 148:2918-22). In addition, a bispecific diabody construct with specificity for C1q also confers CDC activity (Kontermann et al., 1997, *Nat. Biotech.* 15:629-31).

[0166] The in vivo half-life of an antibody can also impact on its effector functions. In some embodiments, it is desir-

able to increase or decrease the half-life of an antibody to modify its therapeutic activities. FcRn is a receptor that is structurally similar to MHC Class I antigen that non-covalently associates with β2-microglobulin. FcRn regulates the catabolism of IgGs and their transcytosis across tissues (Ghetie and Ward, 2000, *Annu. Rev. Immunol.* 18:739-766; Ghetie and Ward, 2002, *Immunol. Res.* 25:97-113). The IgG-FcRn interaction takes place at pH 6.0 (pH of intracellular vesicles) but not at pH 7.4 (pH of blood); this interaction enables IgGs to be recycled back to the circulation (Ghetie and Ward, 2000, *Ann. Rev. Immunol.* 18:739-766; Ghetie and Ward, 2002, *Immunol. Res.* 25:97-113). The region on human IgG_1 involved in FcRn binding has been mapped (Shields et al., 2001, *J. Biol. Chem.* 276:6591-604). Alanine substitutions at positions Pro238, Thr256, Thr307, Gln311, Asp312, Glu380, Glu382, or Asn434 of human IgG_1 enhance FcRn binding (Shields et al., 2001, *J. Biol. Chem.* 276:6591-604). IgG_1 molecules harboring these substitutions are expected to have longer serum half-lives. Consequently, these modified IgG_1 molecules may be able to carry out their effector functions, and hence exert their therapeutic efficacies, over a longer period of time compared to unmodified IgG_1.

V. Assays for Cytotoxic, Cytostatic, and Immunomodulatory Activities

[0167] Methods of determining whether an antibody mediates effector function against a target cell are known. Illustrative examples of such methods are described infra.

[0168] For determining whether an anti-CD70 antibody or derivative mediates antibody-dependent cellular cytotoxicity against activated immune cells or CD70-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 targets 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 $Na_2{}^{51}CrO_4$ 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.

[0169] To determine whether an anti-CD70 antibody or derivative mediates antibody-dependent cellular phagocytosis against activated immune cells or CD70-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.

[0170] 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 a CD70-binding agent mediates CDC on activated immune cells or CD70-expressing cancer cells. Illustrative examples of such methods are described infra.

[0171] The source of active complement can either be normal human serum or purified from laboratory animal including rabbits. In a standard assay, a CD70-binding agent 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 CD70-binding agent to mediate cell lysis can be determined by several readouts. In one example, a $Na^{51}CrO_4$ release assay is used. In this assay, target cells are labeled with $Na^{51}CrO_4$. Unincorporated $Na^{51}CrO_4$ 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 CD70-binding agent 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 $Na^{51}CrO_4$ 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 CD70-binding agents. Percentage cell lysis is calculated as (CD70-binding agent-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 CD70-binding agent with complement and incubated as described above. At the end of incubation, 1/10 volume of Alamar Blue (Biosource International, Camarillo, Calif.) is added. Incubation is continued for up to 16 hours at 37° C.

in a 5% CO_2 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 CD70-binding agent 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.

VI. Animal Models of Immunological Disorders or CD70-Expressing Cancers

[0172] The anti-CD70 binding agents, e.g., antibodies or derivatives, can be tested or validated in animal models of immunological disorders or CD70-expressing cancers. A number of established animal models of immunological disorders or CD70-expressing cancers are known to the skilled artisan, any of which can be used to assay the efficacy of the anti-CD70 antibody or derivative. Non-limiting examples of such models are described infra.

[0173] Examples for animal models of systemic and organ-specific autoimmune diseases including diabetes, lupus, systemic sclerosis, Sjögren's Syndrome, experimental autoimmune encephalomyelitis (multiple sclerosis), thyroiditis, myasthenia gravis, arthritis, uveitis, and inflammatory bowel disease have been described by Bigazzi, "Animal Models of Autoimmunity: Spontaneous and Induced," in The Autoimmune Diseases (Rose and Mackay eds., Academic Press, 1998) and in "Animal Models for Autoimmune and Inflammatory Disease," in *Current Protocols in Immunology* (Coligan et al. eds., Wiley and Sons, 1997).

[0174] Allergic conditions, e.g., asthma and dermatitis, can also be modeled in rodents. Airway hypersensitivity can be induced in mice by ovalbumin (Tomkinson et al., 2001, *J. Immunol.* 166:5792-800) or *Schistosoma mansoni* egg antigen (Tesciuba et al., 2001, *J. Immunol.* 167:1996-2003). The Nc/Nga strain of mice show marked increase in serum IgE and spontaneously develop atopic dermatitis-like lesions (Vestergaard et al., 2000, *Mol. Med. Today* 6:209-10; Watanabe et al., 1997, *Int. Immunol.* 9:461-66; Saskawa et al., 2001, *Int. Arch. Allergy Immunol.* 126:239-47).

[0175] Injection of immuno-competent donor lymphocytes into a lethally irradiated histo-incompatible host is a classical approach to induce GVHD in mice. Alternatively, the parent B6D2F1 murine model provides a system to induce both acute and chronic GVHD. In this model the B6D2F1 mice are F1 progeny from a cross between the parental strains of C57BL/6 and DBA/2 mice. Transfer of DBA/2 lymphoid cells into non-irradiated B6D2F1 mice causes chronic GVHD, whereas transfer of C57BL/6, C57BL/10 or B10.D2 lymphoid cells causes acute GVHD (Slayback et al., 2000, *Bone Marrow Transpl.* 26:931-938; Kataoka et al., 2001, *Immunology* 103:310-318).

[0176] Additionally, both human hematopoietic stem cells and mature peripheral blood lymphoid cells can be engrafted into SCID mice, and these human lympho-hematopoietic cells remain functional in the SCID mice (McCune et al., 1988, *Science* 241:1632-1639; Kamel-Reid and Dick, 1988, *Science* 242:1706-1709; Mosier et al., 1988, *Nature* 335:256-259). This has provided a small animal model system for the direct testing of potential therapeutic agents on human lymphoid cells. (See, e.g., Tournoy et al., 2001, *J. Immunol.* 166:6982-6991).

[0177] Moreover, small animal models to examine the in vivo efficacies of the anti-CD70 antibodies or derivatives can be created by implanting CD70-expressing human tumor cell lines into appropriate immunodeficient rodent strains, e.g., athymic nude mice or SCID mice. Examples of CD70-expressing human lymphoma cell lines include, for example, Daudi (Ghetie et al., 1994, *Blood* 83:1329-36; Ghetie et al., 1990, *Int. J. Cancer* 15:481-85; de Mont et al., 2001, *Cancer Res.* 61:7654-59), HS-Sultan (Caftan and Maung, 1996, *Cancer Chemother. Pharmacol.* 38:548-52; Caftan and Douglas, 1994, *Leuk. Res.* 18:513-22), Raji (Ochakovskaya et al., 2001, *Clin. Cancer Res.* 7:1505-10; Breisto et al., 1999, *Cancer Res.* 59:2944-49), and CA46 (Kreitman et al., 1999, *Int. J. Cancer* 81:148-55). Non-limiting example of a CD70-expressing Hodgkin's lymphoma line is L428 (Drexler, 1993, *Leuk. Lymphoma* 9:1-25; Dewan et al., 2005, *Cancer Sci.* 96:466-473). Non-limiting examples of CD70 expressing human renal cell carcinoma cell lines include 786-O (Ananth et al., 1999, *Cancer Res.* 59:2210-16; Datta et al., 2001, *Cancer Res.* 61:1768-75), ACHN (Hara et al., 2001, *J. Urol.* 166:2491-94; Miyake et al., 2002, *J. Urol.* 167:2203-08), Caki-1 (Prewett et al., 1998, *Clin. Cancer Res.* 4:2957-66; Shi and Siemann, 2002, *Br. J. Cancer* 87:119-26), and Caki-2 (Zellweger et al., 2001, *Neoplasia* 3:360-67). Non-limiting examples of CD70-expressing nasopharyngeal carcinoma cell lines include C15 and C17 (Busson et al., 1988, *Int. J. Cancer* 42:599-606; Bernheim et al., 1993, *Cancer Genet. Cytogenet.* 66:11-5). Non-limiting examples of CD70-expressing human glioma cell lines include U373 (Palma et al., 2000, *Br. J. Cancer* 82:480-7) and U87MG (Johns et al., 2002, *Int. J. Cancer* 98:398-408). Non-limiting examples of multiple myeloma cell lines include MM.1S (Greenstein et al., 2003, *Experimental Hematology* 31:271-282) and L363 (Diehl et al., 1978, *Blut* 36:331-338). (See also Drexler and Matsuo, 2000, *Leukemia Research* 24:681-703). 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. Once established within a host, these tumor models can be applied to evaluate the therapeutic efficacies of the anti-CD70 antibody or derivatives as described herein on modulating in vivo tumor growth.

VII. CD70-Associated Disorders

[0178] The anti-CD70 binding agents (e.g., antibodies and derivatives) as described herein are useful for treating or preventing a CD70-expressing cancer or an immunological disorder characterized by expression of CD70 by inappropriate activation of immune cells (e.g., lymphocytes or dendritic cells). Such expression of CD70 can be due to, for example, increased CD70 protein levels on the cells surface and/or altered antigenicity of the expressed CD70. Treatment or prevention of the immunological disorder, according to the methods described herein, is achieved by administering to a subject in need of such treatment or prevention an effective amount of the anti-CD70 antibody or derivative, whereby the antibody or derivative (i) binds to activated immune cells that express CD70 and that are associated with

the disease state and (ii) exerts a cytotoxic, cytostatic, or immunomodulatory effect on the activated immune cells. In some embodiments, the cytotoxic, cytostatic, or immunomodulatory is exerted without conjugation to a cytotoxic, cytostatic, or immunomodulatory agent. In some embodiments, the cytotoxic, cytostatic, or immunomodulatory is exerted by conjugation to a cytotoxic, cytostatic, or immunomodulatory agent.

[0179] Immunological diseases that are characterized by inappropriate activation of immune cells and that can be treated or prevented by the methods described herein can be classified, for example, by the type(s) of hypersensitivity reaction(s) that underlie the disorder. These reactions are typically classified into four types: anaphylactic reactions, cytotoxic (cytolytic) reactions, immune complex reactions, or cell-mediated immunity (CMI) reactions (also referred to as delayed-type hypersensitivity (DTH) reactions). (See, e.g., *Fundamental Immunology* (William E. Paul ed., Raven Press, N.Y., 3rd ed. 1993).)

[0180] Specific examples of such immunological diseases include the following: rheumatoid arthritis, psoriatic arthritis, autoimmune demyelinative diseases (e.g., multiple sclerosis, allergic encephalomyelitis), endocrine ophthalmopathy, uveoretinitis, systemic lupus erythematosus, myasthenia gravis, Grave's disease, glomerulonephritis, autoimmune hepatological disorder, inflammatory bowel disease (e.g., Crohn's disease), anaphylaxis, allergic reaction, Sjogren's syndrome, type I diabetes mellitus, primary biliary cirrhosis, Wegener's granulomatosis, fibromyalgia, polymyositis, dermatomyositis, multiple endocrine failure, Schmidt's syndrome, autoimmune uveitis, Addison's disease, adrenalitis, thyroiditis, Hashimoto's thyroiditis, autoimmune thyroid disease, pernicious anemia, gastric atrophy, chronic hepatitis, lupoid hepatitis, atherosclerosis, subacute cutaneous lupus erythematosus, hypoparathyroidism, Dressler's syndrome, autoimmune thrombocytopenia, idiopathic thrombocytopenic purpura, hemolytic anemia, pemphigus vulgaris, pemphigus, dermatitis herpetiformis, alopecia arcata, pemphigoid, scleroderma, progressive systemic sclerosis, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia), male and female autoimmune infertility, ankylosing spondolytis, ulcerative colitis, mixed connective tissue disease, polyarteritis nedosa, systemic necrotizing vasculitis, atopic dermatitis, atopic rhinitis, Goodpasture's syndrome, Chagas' disease, sarcoidosis, rheumatic fever, asthma, recurrent abortion, anti-phospholipid syndrome, farmer's lung, erythema multiforme, post cardiotomy syndrome, Cushing's syndrome, autoimmune chronic active hepatitis, bird-fancier's lung, toxic epidermal necrolysis, Alport's syndrome, alveolitis, allergic alveolitis, fibrosing alveolitis, interstitial lung disease, erythema nodosum, pyoderma gangrenosum, transfusion reaction, Takayasu's arteritis, polymyalgia rheumatica, temporal arteritis, schistosomiasis, giant cell arteritis, ascariasis, aspergillosis, Sampter's syndrome, eczema, lymphomatoid granulomatosis, Behcet's disease, Caplan's syndrome, Kawasaki's disease, dengue, encephalomyelitis, endocarditis, endomyocardial fibrosis, endophthalmitis, erythema elevatum et diutinum, psoriasis, erythroblastosis fetalis, eosinophilic faciitis, Shulman's syndrome, Felty's syndrome, filariasis, cyclitis, chronic cyclitis, heterochronic cyclitis, Fuch's cyclitis, IgA nephropathy, Henoch-Schonlein purpura, graft versus host disease, transplantation rejection, cardiomyopathy, Eaton-Lambert syndrome, relapsing polychondritis, cryoglobulinemia, Waldenstrom's macroglobulemia, Evan's syndrome, and autoimmune gonadal failure.

[0181] Accordingly, the methods described herein encompass treatment of disorders of B lymphocytes (e.g., systemic lupus erythematosus, Goodpasture's syndrome, rheumatoid arthritis, and type I diabetes), Th_1-lymphocytes (e.g., rheumatoid arthritis, multiple sclerosis, psoriasis, Sjorgren's syndrome, Hashimoto's thyroiditis, Grave's disease, primary biliary cirrhosis, Wegener's granulomatosis, tuberculosis, or graft versus host disease), or Th_2-lymphocytes (e.g., atopic dermatitis, systemic lupus erythematosus, atopic asthma, rhinoconjunctivitis, allergic rhinitis, Omenn's syndrome, systemic sclerosis, or chronic graft versus host disease). Generally, disorders involving dendritic cells involve disorders of Th_1-lymphocytes or Th_2-lymphocytes.

[0182] In some embodiments, the immunological disorder is a T cell-mediated immunological disorder, such as a T cell disorder in which activated T cells associated with the disorder express CD70. Anti-CD70 binding agents (e.g., antibodies or derivatives) can be administered to deplete such CD70-expressing activated T cells. In a specific embodiment, administration of anti-CD70 antibodies or derivatives can deplete CD70-expressing activated T cells, while resting T cells are not substantially depleted by the anti-CD70 or derivative. In this context, "not substantially depleted" means that less than about 60%, or less than about 70% or less than about 80% of resting T cells are not depleted.

[0183] The anti-CD70 binding agents (e.g., antibodies and derivatives) are also useful for treating or preventing a CD70-expressing cancer. Treatment or prevention of a CD70-expressing cancer, according to the methods described herein, is achieved by administering to a subject in need of such treatment or prevention an effective amount of the anti-CD70 antibody or derivative, whereby the antibody or derivative (i) binds to CD70-expressing cancer cells and (ii) exerts a cytotoxic or cytostatic effect to deplete or inhibit the proliferation of the CD70-expressing cancer cells. In some embodiments, the cytotoxic, cytostatic, or immunomodulatory is exerted without conjugation to a cytotoxic, cytostatic, or immunomodulatory agent. In some embodiments, the cytotoxic, cytostatic, or immunomodulatory is exerted by conjugation to a cytotoxic, cytostatic, or immunomodulatory agent.

[0184] CD70-expressing cancers that can be treated or prevented by the methods described herein include, for example, different subtypes of Non-Hodgkin's Lymphoma (indolent NHLs, follicular NHLs, small lymphocytic lymphomas, lymphoplasmacytic NHLs, or marginal zone NHLs); Hodgkin's disease (e.g., Reed-Sternberg cells); cancers of the B-cell lineage, including, e.g., diffuse large B-cell lymphomas, follicular lymphomas, Burkitt's lymphoma, mantle cell lymphomas, B-cell lymphocytic leukemias (e.g., acute lymphocytic leukemia, chronic lymphocytic leukemia); Epstein Barr Virus positive B cell lymphomas; renal cell carcinomas (e.g., clear cell and papillary); nasopharyngeal carcinomas; thymic carcinomas; gliomas; glioblastomas; neuroblastomas; astrocytomas; meningiomas; Waldenstrom macroglobulinemia; multiple myelomas; and colon, stomach, and rectal carcinomas. The cancer can be, for example, newly diagnosed, pre-treated or refractory or relapsed. In some embodiments, a CD70-expressing cancer

has at least about 15,000, at least about 10,000 or at least about 5,000 CD70 molecules/cell.

VII. Pharmaceutical Compositions Comprising Anti-CD70 Antibodies and Derivatives and Administration Thereof

[0185] A composition comprising a CD70 binding agent (e.g., an anti-CD70 antibody or derivative) can be administered to a subject having or at risk of having an immunological disorder or a CD70-expressing cancer. The invention further provides for the use of a CD70 binding agent (e.g., an anti-CD70 antibody or derivative) in the manufacture of a medicament for prevention or treatment of a CD70 expressing cancer or immunological disorder. 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 or derivatives can be administered either alone or in combination with other compositions in the prevention or treatment of the immunological disorder or CD70-expressing cancer.

[0186] Various delivery systems are known and can be used to administer the CD70 binding agent. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The CD70 binding agent 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.

[0187] In specific embodiments, the CD70 binding agent 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 binding agent does not absorb are used.

[0188] In other embodiments, the anti-CD70 binding agent is delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, 1990, *Science* 249:1527-1533; Sefton, 1989, *CRC Crit. Ref. Biomed. Eng.* 14:201; Buchwald et al., 1980, *Surgery* 88:507; Saudek et al., 1989, *N. Engl. J. Med.* 321:574). In another embodiment, polymeric materials can be used. (See Medical Applications of Controlled Release (Langer and Wise eds., CRC Press, Boca Raton, Fla., 1974); Controlled Drug Bioavailability, Drug Product Design and Performance (Smolen and Ball eds., Wiley, New York, 1984); Ranger and Peppas, 1983, *Macromol. Sci. Rev. Macromol. Chem.* 23:61. See also Levy et al., 1985, *Science* 228:190; During et al., 1989, *Ann. Neurol.* 25:351; Howard et al., 1989, *J Neurosurg.* 71:105.) Other controlled release systems are discussed, for example, in Langer, supra.

[0189] A CD70 binding agent (e.g., an anti-CD70 antibody or derivative) can be administered as pharmaceutical compositions comprising a therapeutically effective amount of the binding agent 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.

[0190] 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.

[0191] Further, the pharmaceutical composition can be provided as a pharmaceutical kit comprising (a) a container containing a CD70 binding agent (e.g., an anti-CD70 antibody or derivative) 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 derivative. 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.

[0192] The amount of the CD70 binding agent (e.g., anti-CD70 antibody or derivative) that is effective in the

treatment or prevention of an immunological disorder or CD70-expressing 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 immunological disorder or CD70-expressing 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.

[0193] For example, toxicity and therapeutic efficacy of the anti-CD70 antibody or derivative can be determined in cell cultures or experimental animals by standard pharmaceutical procedures for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (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 LD_{50}/ED_{50}. A CD70-binding agent (e.g., an anti-CD70 antibody or derivative) that exhibits a large therapeutic index is preferred. Where a CD70-binding agent exhibits toxic side effects, a delivery system that targets the CD70-binding agent to the site of affected tissue can be used to minimize potential damage non-CD70-expressing cells and, thereby, reduce side effects.

[0194] 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 CD70 binding agent typically lies within a range of circulating concentrations that include the ED_{50} 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 a CD70 binding agent 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 IC_{50} (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.

[0195] Generally, the dosage of an anti-CD70 antibody or derivative administered to a patient with an immunological disorder or CD70-expressing cancer is about 0.1 mg/kg to 100 mg/kg of the subject's body weight. More typically, the dosage 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. 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 or derivative comprising humanized or chimeric antibodies and less frequent administration is often possible.

[0196] A dose of an anti-CD70 binding agent 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.

[0197] In some embodiments, the dosage of an anti-CD70 binding agent corresponds to a sub-optimal dosage (i.e., below the EC_{50} for the anti-CD70 binding agent (e.g., an antibody drug conjugate). For example, the dosage of an anti-CD70 binding agent 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.

[0198] In some embodiments, the dosage of an anti-CD70 binding agent (e.g., an antibody drug conjugate) 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 binding agent can be administered from 1 to 10 times per four day, five day, six day or seven day period.

[0199] In some embodiments, the pharmaceutical compositions comprising the CD70 binding agent 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 binding agent also can be co-administered in combination with one or more therapeutic agents for the treatment or prevention of immunological disorders or CD70-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 or immunological disorders). Combination therapy can also include, e.g., administration of an agent that targets a receptor or receptor complex other than CD70 on the surface of activated lymphocytes, dendritic cells or CD70-expressing cancer cells. An example of such an agent includes a second, non-CD70 antibody that binds to a molecule at the surface of an activated lymphocyte, dendritic cell or CD70-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 cancer cell and enhances the cytotoxic or cytostatic effect of the anti-CD70 antibody by delivering a cytostatic or cytotoxic signal to the activated lymphocyte, dendritic cell or CD70-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).

[0200] With respect to therapeutic regimens for combinatorial administration, in a specific embodiment, an anti-CD70 binding agent is administered concurrently with a therapeutic agent. In another specific embodiment, the therapeutic agent is administered prior or subsequent to administration of the anti-CD70 antibody or derivative, 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 or derivative. In some embodiments, the subject is monitored following administration of the anti-CD70 binding agent, and optionally the therapeutic agent.

[0201] The therapeutic agent can be, for example, any agent that exerts a therapeutic effect on cancer cells or activated immune cells. Typically, the therapeutic agent is a cytotoxic or immunomodulatory agent. 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).

[0202] 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 cisplatin, 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.

[0203] Individual cytotoxic or immunomodulatory agents include, for example, an androgen, anthramycin (AMC), asparaginase, 5-azacytidine, azathioprine, bleomycin, busulfan, buthionine sulfoximine, camptothecin, carboplatin, carmustine (BSNU), CC-1065, chlorambucil, cisplatin, colchicine, cyclophosphamide, cytarabine, cytidine arabinoside, cytochalasin B, dacarbazine, dactinomycin (actinomycin), daunorubicin, decarbazine, docetaxel, doxorubicin, an estrogen, 5-fluordeoxyuridine, 5-fluorouracil, gramicidin D, hydroxyurea, idarubicin, ifosfamide, irinotecan, lomustine (CCNU), mechlorethamine, melphalan, 6-mercaptopurine, methotrexate, mithramycin, mitomycin C, mitoxantrone, nitroimidazole, paclitaxel, plicamycin, procarbizine, rapamycin (Sirolimus), streptozotocin, tenoposide, 6-thioguanine, thioTEPA, topotecan, vinblastine, vincristine, vinorelbine, VP-16 and VM-26.

[0204] 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.

[0205] In some embodiments, the cytotoxic agent is a conventional chemotherapeutic such as, for example, doxorubicin, paclitaxel, melphalan, vinca alkaloids, methotrexate, mitomycin C or etoposide. In some embodiments, the therapeutic agent can be a combined therapy, such as CHOP (Cyclophosphamide, Doxorubicin, Prednisolone and Vincristine), CHOP-R (Cyclophosphamide, Doxorubicin Vincristine, Prednisolone, and rituximab) or ABVD (Doxorubicin, Bleomycin, Vinblastine and Dacarbazine). Agents such as CC-1065 analogues, calicheamicin, maytansine, analogues of dolastatin 10, rhizoxin, and palytoxin can be linked to the anti-CD70 antibodies or derivatives thereof.

[0206] 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. Pat. 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.

[0207] In specific embodiments, the cytotoxic agent is a DNA minor groove binding agent. (See, e.g., U.S. Pat. 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).

[0208] Examples of anti-tubulin agents include, but are not limited to, taxanes (e.g., Taxol® (paclitaxel), Taxotere® (docetaxel)), T67 (Tularik), vinca alkyloids (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.

[0209] 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).

[0210] In some embodiments, the therapeutic agent is not a radioisotope. In some embodiments, the therapeutic agent is not ricin or saporin.

[0211] In certain embodiments, the therapeutic agent is an anti-VEGF agent, such as AVASTIN (bevacizumab) or NEXAVAR (Sorafenib); a PDGF blocker, such as SUTENT (sunitinib malate); or a kinase inhibitor, such as NEXAVAR (sorafenib tosylateor).

[0212] In some embodiments, the cytotoxic or immunomodulatory agent is an antimetabolite. The antimetabolite can be, for example, a purine antagonist (e.g., azothioprine or mycophenolate mofetil), a dihydrofolate reductase inhibitor (e.g., methotrexate), acyclovir, gangcyclovir, zidovudine, vidarabine, ribavarin, azidothymidine, cytidine arabinoside, amantadine, dideoxyuridine, iododeoxyuridine, poscarnet, or trifluridine.

[0213] In other embodiments, the cytotoxic or immunomodulatory agent is tacrolimus, cyclosporine or rapamycin. In further embodiments, the cytoxic agent is aldesleukin, alemtuzumab, alitretinoin, allopurinol, altretamine, amifostine, anastrozole, arsenic trioxide, bexarotene, bexarotene, calusterone, capecitabine, celecoxib, cladribine, Darbepoetin alfa, Denileukin diftitox, dexrazoxane, dromostanolone propionate, epirubicin, Epoetin alfa, estramustine, exemestane, Filgrastim, floxuridine, fludarabine, fulvestrant, gemcitabine, gemtuzumab ozogamicin, goserelin, idarubicin, ifosfamide, imatinib mesylate, Interferon alfa-2a, irinotecan, letrozole, leucovorin, levamisole, mechlorethamine or nitrogen mustard, megestrol, mesna, methotrexate, methoxsalen, mitomycin C, mitotane, nandrolone phenpropionate, oprelvekin, oxaliplatin, pamidronate, pegademase, pegaspargase, pegfilgrastim, pentostatin, pipobroman, plicamycin, porfimer sodium, procarbazine, quinacrine, rasburicase, Sargramostim, streptozocin, tamoxifen, temozolomide, teniposide, testolactone, thioguanine,

toremifene, Tositumomab, Trastuzumab, tretinoin, uracil mustard, valrubicin, vinblastine, vincristine, vinorelbine or zoledronate.

[0214] In additional embodiments, the therapeutic agent is an antibody, such as a humanized anti HER2 monoclonal antibody, RITUXAN (rituximab; Genentech; a chimeric anti CD20 monoclonal antibody); OVAREX (AltaRex Corporation, MA); PANOREX (Glaxo Wellcome, NC; a murine IgG2a antibody); Cetuximab Erbitux (Imclone Systems Inc., NY; an anti-EGFR IgG chimeric antibody); Vitaxin (MedImmune, Inc., MD; Campath I/H (Leukosite, MA; a humanized IgG1 antibody); Smart MI95 (Protein Design Labs, Inc., CA; a humanized anti-CD33 IgG antibody); LymphoCide (Immunomedics, Inc., NJ; a humanized anti-CD22 IgG antibody); Smart ID10 (Protein Design Labs, Inc., CA; a humanized anti-HLA-DR antibody); Oncolym (Techniclone, Inc., CA; a radiolabeled murine anti-HLA-Dr10 antibody); Allomune (BioTransplant, CA; a humanized anti-CD2 mAb); Avastin (Genentech, Inc., CA; an anti-VEGF humanized antibody); Epratuzamab (Immunomedics, Inc., NJ and Amgen, CA; an anti-CD22 antibody); CEAcide (Immunomedics, NJ; a humanized anti-CEA antibody); or an anti-CD40 antibody (e.g., as disclosed in U.S. Pat. No. 6,838,261).

[0215] Other suitable antibodies include, but are not limited to, antibodies against the following antigens: CA125, CA15-3, CA19-9, L6, Lewis Y, Lewis X, alpha fetoprotein, CA 242, placental alkaline phosphatase, prostate specific membrane antigen, prostatic acid phosphatase, epidermal growth factor, MAGE-1, MAGE-2, MAGE-3, MAGE-4, anti-transferrin receptor, p97, MUC1-KLH, CEA, gp100, MART1, Prostate Specific Antigen, IL-2 receptor, CD20, CD52, CD30, CD33, CD22, human chorionic gonadotropin, CD38, CD40, mucin, P21, MPG, and Neu oncogene product.

[0216] In some embodiments, the therapeutic agent is an immunomodulatory agent. The immunomodulatory agent can be, for example, gancyclovir, etanercept, tacrolimus, cyclosporine, rapamycin, REVLIMID (lenalidomide), cyclophosphamide, azathioprine, mycophenolate mofetil or methotrexate. Alternatively, the immunomodulatory agent can be, for example, a glucocorticoid (e.g., cortisol or aldosterone) or a glucocorticoid analogue (e.g., prednisone or dexamethasone).

[0217] In some typical embodiments, the immunomodulatory agent is an anti-inflammatory agent, such as arylcarboxylic derivatives, pyrazole-containing derivatives, oxicam derivatives and nicotinic acid derivatives. Classes of anti-inflammatory agents include, for example, cyclooxygenase inhibitors, 5-lipoxygenase inhibitors, and leukotriene receptor antagonists. In some embodiments, the immodulatory agent is a cytokine, such as G-CSF, GM-CSF or IL-2.

[0218] Suitable cyclooxygenase inhibitors include meclofenamic acid, mefenamic acid, carprofen, diclofenac, diflunisal, fenbufen, fenoprofen, ibuprofen, indomethacin, ketoprofen, nabumetone, naproxen, sulindac, tenoxicam, tolmetin, and acetylsalicylic acid.

[0219] Suitable lipoxygenase inhibitors include redox inhibitors (e.g., catechol butane derivatives, nordihydroguaiaretic acid (NDGA), masoprocol, phenidone, lanopalen, indazolinones, naphazatrom, benzofuranol, alkylhydroxylamine), and non-redox inhibitors (e.g., hydroxythiazoles, methoxyalkylthiazoles, benzopyrans and derivatives thereof, methoxytetrahydropyran, boswellic acids and acetylated derivatives of boswellic acids, and quinolinemethoxyphenylacetic acids substituted with cycloalkyl radicals), and precursors of redox inhibitors.

[0220] Other suitable lipoxygenase inhibitors include antioxidants (e.g., phenols, propyl gallate, flavonoids and/or naturally occurring substrates containing flavonoids, hydroxylated derivatives of the flavones, flavonol, dihydroquercetin, luteolin, galangin, orobol, derivatives of chalcone, 4,2',4'-trihydroxychalcone, ortho-aminophenols, N-hydroxyureas, benzofuranols, ebselen and species that increase the activity of the reducing selenoenzymes), iron chelating agents (e.g., hydroxamic acids and derivatives thereof, N-hydroxyureas, 2-benzyl-1-naphthol, catechols, hydroxylamines, carnosol trolox C, catechol, naphthol, sulfasalazine, zyleuton, 5-hydroxyanthranilic acid and 4-(omega-arylalkyl)phenylalkanoic acids), imidazole-containing compounds (e.g., ketoconazole and itraconazole), phenothiazines, and benzopyran derivatives.

[0221] Yet other suitable lipoxygenase inhibitors include inhibitors of eicosanoids (e.g., octadecatetraenoic, eicosatetraenoic, docosapentaenoic, eicosahexaenoic and docosahexaenoic acids and esters thereof, PGE1 (prostaglandin E1), PGA2 (prostaglandin A 2), viprostol, 15-monohydroxyeicosatetraenoic, 15-monohydroxy-eicosatrienoic and 15-monohydroxyeicosapentaenoic acids, and leukotrienes B5, C5 and D5), compounds interfering with calcium flows, phenothiazines, diphenylbutylamines, verapamil, fuscoside, curcumin, chlorogenic acid, caffeic acid, 5,8,11,14-eicosatetrayenoic acid (ETYA), hydroxyphenylretinamide, Ionapalen, esculin, diethylcarbamazine, phenantroline, baicalein, proxicromil, thioethers, diallyl sulfide and di-(1-propenyl) sulfide.

[0222] Leukotriene receptor antagonists include calcitriol, ontazolast, Bayer Bay-x-1005, Ciba-Geigy CGS-25019C, ebselen, Leo Denmark ETH-615, Lilly LY-293111, Ono ONO-4057, Terumo TMK-688, Boehringer Ingleheim BI-RM-270, Lilly LY 213024, Lilly LY 264086, Lilly LY 292728, Ono ONO LB457, Pfizer 105696, Perdue Frederick PF 10042, Rhone-Poulenc Rorer RP 66153, SmithKline Beecham SB-201146, SmithKline Beecham SB-201993, SmithKline Beecham SB-209247, Searle SC-53228, Sumitamo SM 15178, American Home Products Way 121006, Bayer Bay-o-8276, Warner-Lambert CI-987, Warner-Lambert CI-987BPC-15LY 223982, Lilly LY 233569, Lilly LY-255283, MacroNex MNX-160, Merck and Co. MK-591, Merck and Co. MK-886, Ono ONO-LB-448, Purdue Frederick PF-5901, Rhone-Poulenc Rorer RG 14893, Rhone-Poulenc Rorer RP 66364, Rhone-Poulenc Rorer RP 69698, Shionoogi S-2474, Searle SC-41930, Searle SC-50505, Searle SC-51146, Searle SC-52798, SmithKline Beecham SKandF-104493, Leo Denmark SR-2566, Tanabe T-757 and Teijin TEI-1338.

[0223] The invention is further described in the following examples, which are in not intended to limit the scope of the invention. Cell lines described in the following examples were maintained in culture according to the conditions specified by the American Type Culture Collection (ATCC) or Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH, Braunschweig, Germany (DMSZ), or as otherwise known. Cell culture reagents were obtained from Invitrogen Corp., Carlsbad, Calif.

Example 1: Production of Humanized Anti-CD70 Antibody Variants

[0224] The nucleotide and amino acid sequences of the heavy and light variable regions of antiCD70 murine monoclonal antibody, 1F6, and a chimeric variant of 1F6, c1F6, are set forth as SEQ ID NOS:1, 2, 21 and 22, respectively. (See also U.S. Patent Application No. 60/645,355, filed Jan. 19, 2005). Human acceptor sequences for humanization of c1F6 were chosen from human germline exon V_H, J_H, V_κ and J_κ sequences. Acceptor sequences for c1F6 V_H domain humanization were chosen from germline V_H exons V_H1-18 (Matsuda et al., 1993, *Nature Genetics* 3:88-94) or V_H1-2 (Shin et al., 1991, *EMBO J.* 10:3641-3645) and J_H exon J_H-6 (Manila et al., 1995, *Eur. J. Immunol.* 25:2578-2582). Germline V_κ exon B3 (Cox et al., 1994, *Eur. J. Immunol.* 24:827-836) and J_κ exon Jκ-1 (Hieter et al., 1982, *J Biol. Chem.* 257:1516-1522) were chosen as acceptor sequences for c1F6 V_L domain humanization. 1F6 murine CDRs, determined according to the Kabat definition, were grafted onto the chosen human germline template. Briefly, synthetic overlapping oligonucleotides spanning the humanized V_H or V_L domain were generated and PCR overlap extension was used to assemble each domain. Restriction sites incorporated into the PCR product were used to directionally clone the V_H and V_L domain into a pCMV expression vector in frame with human IgG1 constant domains or Kappa constant domain, respectively.

[0225] Several framework positions were chosen for reintroduction of mouse donor residues. These were positions H46, H67, H68, H69, H70, H71, H80, H81, H82, H82A and H91 in the V_H domain, according to the Kabat numbering convention. No framework positions were altered in the V_L domain, although mouse CDR1 residues at positions L25 and L33 were chosen for introduction of the human acceptor residue for that position.

[0226] Several variants of humanized 1F6 were generated by incorporating different combinations of mouse framework donor residues in the V_H domain or human CDR residues in the V_L domain. These variants are summarized in Tables 2 and 3 below.

TABLE 2

V_H variant	VH exon acceptor sequence	donor framework residues
hV_HA	VH1-18	H71, H91
hV_HB	VH1-18	H71
hV_HC	VH1-18	H91
hV_HD	VH1-18	none
hV_HE	VH1-2	none
hV_HF	VH1-18	H67, H68, H69, H70, H71
hV_HG	VH1-18	H80, H81, H82, H82A
hV_HH	VH1-18	H67, H68, H69, H70, H71, H80, H81, H82, H82A
hV_HI	VH1-18	H46, H71, H91
hV_HJ	VH1-2	H46
hV_HK	VH1-2	H71
hV_HL	VH1-2	H46, H71
hV_HM	VH1-18	H46, H67, H68, H69, H70, H71
hV_HN	VH1-18	H69, H70, H71, H80

TABLE 3

V_L variant	Acceptor CDR residue
hV_LA	none
hV_LB	L25
hV_LC	L33
hV_LD	L25, L33

[0227] The differences between some of the humanized variants with the murine and human V_H sequences are illustrated in FIGS. **1** and **2**. An alignment of humanized 1F6 V_H variants hV_HE and hV_HJ with 1F6 mV_H and human germline V_H exon V_H1-2 and J_H exon JH6 is shown in FIG. **1**. An alignment of humanized 1F6 V_H variants hV_HH and hV_HM with 1F6 mV_H and human germline V_H exon V_H1-18 and J_H exon J_H6 is shown in FIG. **2**. An alignment of humanized 1F6 V_L variant hV_LA with 1F6 mV_L and human germline V_κ exon B3 and J_κ exon Jκ-1 is shown in FIG. **3**.

Example 2: Binding Affinities of Humanized 1F6 Variants

[0228] Humanized 1F6 variants HDLA (hV_HD and hV_LA), HHLA (hV_HH and hV_LA), and HJLA (hV_HJ and hV_LA), were selected for binding affinity analysis. One mg of each humanized antibody and c1F6 were transiently expressed in 293 cells and labeled with europium using the Eu-N1 iodoacetamido chelate (Perkin Elmer). Saturation binding to a panel of CD70 positive cell lines was assessed for each labeled antibody. The cell lines selected were ACHN, Caki-2, Caki-1, and 786-O with antigen copies/cell determined by quantitative flow cytometry (or fluorescence activated cell sorting, i.e., FACS) of 30,000, 99,000, 235,000, and 252,000 respectively.

[0229] Europium-labeled antibodies were incubated with cells for 1 hour at 4° C. over a range of concentrations in 96 well plates. Following incubation europium was released by resuspension of the cells in Enhancement Buffer (Perkin Elmer). Fluorescence was read in a Fusion HT plate reader using a top detector format and excitation of 335 nm and emission of 620 nm. Data was fit to a one binding site hyperbola using GraphPad Prism 4. The results are shown below in Table 4.

TABLE 4

		Apparent binding affinity K_D (nM)			
Cell line	Antigen/cell	c1F6	h1F6 HDLA	h1F6 HHLA	h1F6 HJLA
ACHN	30,000	0.30	1.44	0.29	0.68
Caki-1	235,000	1.28	1.29	1.22	1.36
Caki-2	99,000	0.26	0.86	0.15	0.37
786-O	252,000	0.56	0.55	0.28	0.46

[0230] The K_D values for the humanized variants are very similar to c1F6 on all of the cell lines tested, confirming that the humanization process did not significantly reduce antigen binding activity.

Example 3: ADCC Activity of Humanized 1F6

[0231] The ability of humanized 1F6 antibody variants to mediate ADCC against the $CD70^+$ cell lines WIL2-S, 786-O and 769-P was measured using a standard ^{51}Cr release assay. The HHLA, HJLA and HELA variants of humanized 1F6

lysed WIL-2S target cells equivalently and in a dose dependent manner. In contrast, tumor cells treated with CD70-binding murine 1F6 (m1F6) or non-binding control human Ig (hIg) were not killed (FIG. **4**A). Similarly, humanized 1F6 mediated the lysis of two renal cell carcinoma targets in a manner comparable to chimeric 1F6 (FIG. **4**B).

Example 4: CDC Activity of Humanized 1F6

[0232] The ability of humanized 1F6 to mediate CDC was examined using a multiple myeloma cell line (LP-1) and two lymphoma cell lines (MHH PreB-1 and WIL2-S). Target cells were treated with graded doses of chimeric 1F6, humanized 1F6 HJLA or a non-binding human Ig control in the presence of normal human serum. After incubation at 37° C. for 2 hours, lysed cells were identified by flow cytometry after the addition of propidium iodide (5 μg/mL). Cells stained with propidium iodide were considered to have lost plasma membrane integrity as a result of antibody-mediated complement activation and formation of the membrane attack complex. Using this assay, chimeric 1F6 and humanized 1F6 mediated dose-dependent lysis of each target in an equivalent manner (FIG. **5**).

Example 5: ADCP Activity of Humanized 1F6

[0233] The ability of humanized 1F6 to mediate phagocytosis was examined using the $CD70^+$ renal cell carcinoma line 786-O pre-labeled with a red fluorescent membrane dye. Target cells were treated with graded doses of chimeric 1F6, humanized 1F6 HJLA or a non-binding human Ig control and were then mixed with macrophages generated from adherent peripheral blood monocytes cultured in GM-CSF. After incubation at 37° C. for 1 hour, the macrophages were detected with a green fluorescent antibody to the macrophage cell surface marker CD11b. Macrophages that had phagocytosed tumor cells were identified by dual red and green fluorescence as detected by flow cytometry. The presence of tumor cells within macrophages in the dual-positive population was confirmed by fluorescence microscopy. As shown in FIG. **6**, chimeric and humanized 1F6 facilitated phagocytosis of target cells in an antibody-dose dependent fashion and to an equivalent degree. In contrast, target cells incubated with a non-binding control antibody were minimally engulfed by macrophages.

Example 6: In Vitro Cytotoxicity Activity of Humanized 1F6 Variant Drug Conjugates

[0234] Humanized 1F6 variants HELA (hV_HE and hV_LA), HHLA, HJLA, and HMLA (hV_HM and hV_LA), and c1F6 were transiently expressed in 293 cells and conjugated to vcMMAF (described in U.S. Ser. No. 10/983,340; published as U.S. Patent Publication No. 2005-0238649, Oct. 27, 2005) at a loading level of an average of eight drug units per antibody. The resulting conjugates, h1F6 HELA-F8, h1F6 HHLA-F8, h1F6 HJLA-F8, h1F6 HMLA-F8, and c1F6-F8 were tested for cytotoxicity against two CD70 expressing cell lines, 786-O and Caki-1. The conjugates were incubated with the cells for 92 hours, followed by addition of 50 μM resazurin. After a 4 hour incubation period, dye reduction was measured using a Fusion HT fluorescent plate reader (Packard Instruments, Meriden, Conn.). The results of triplicate sampling are shown below in Table 5. The IC_{50} values of all four humanized variants are active within two-fold of c1F6 on both cell lines tested with a potency ranking of c1F6-F8>h1F6 HHLA-F8>h1F6 HMLA-F8>h1F6 HJLA-F8>h1F6 HELA-F8.

TABLE 5

h1F6-vcMMAF	No. of mouse FR residues	Caki-1 IC50 [ng/mL]	786-O IC50 [ng/mL]
h1F6 HELA-F8	0	3.4 (mean = 2.87, n = 3)	5.2 (mean = 3.9, n = 3)
h1F6 HHLA-F8	9	1.4 (mean = 1.87, n = 3)	2.3 (mean = 1.93, n = 3)
h1F6 HJLA-F8	1	2.2 (mean = 2.3, n = 3)	3.4 (mean = 3.03, n = 3)
h1F6 HMLA-F8	6	1.8 (mean = 2.07, n = 3)	2.8 (mean = 2.03, n = 3)
c1F6(293)-F8	0	1.8 (mean = 2.17, n = 3)	2.4 (mean = 1.45, n = 3)

Example 7: In Vivo Screening of Humanized 1F6 Drug Conjugates

[0235] Humanized 1F6 variants HDLA, HHLA, HJLA, and HELA, were transiently expressed in 293 cells and conjugated to mcMMAF (described in U.S. Ser. No. 10/983,340; published as U.S. Patent Publication No. 2005-0238649, Oct. 27, 2005) at a loading level of eight drug units per antibody. An efficacy study of a single dose at 3 mg/kg or 10 mg/kg was performed in a 786-O renal cell carcinoma solid tumor model in nude mice. Tumor volume was measured regularly for 80 days post-tumor implant. The results indicate that tumor volume was greatly reduced in all treated mice in comparison to untreated mice, and all humanized 1F6 variants conjugated to mcMMAF were comparable in efficacy to c1F6mcMMAF.

Example 8: In Vivo Activity of Humanized 1F6 in SCID Mouse Xenograft Models of Disseminated Lymphoma and Multiple Myeloma

[0236] The in vivo antitumor activity of humanized 1F6 (HJLA) was examined in disseminated lymphoma and multiple myeloma xenograft mouse models. To establish disseminated disease, 1×10^6 Raji or 1×10^7 MM1.S or L363 cells were injected into the lateral tail vein of C.B.-17 SCID mice. Mice were dosed with humanized 1F6 (HJLA) or control non-binding antibody by intraperitoneal (i.p.) injection every four days for a total of six doses (Raji) or by intravenous injection into the lateral tail vein once weekly for a total of four weeks (MM.1S and L363) starting one day after cell implant. Disease requiring euthanasia was manifested by hunched posture and lack of grooming, weight loss, cranial swelling and hind limb paralysis, or, in L363-bearing mice, the development of palpable lymphoid tissue-associated tumors.

[0237] The results show that, in each tumor model (FIGS. **7**A, **7**B and **7**C), survival of mice treated with humanized 1F6 was significantly prolonged compared to that of untreated mice or mice receiving non-binding control antibody. The effect of humanized 1F6 treatment was further evaluated in multiple myeloma xenografts (L363 and MM.1S cells) by measuring the level of tumor-derived monoclonal protein (λ light chain) in the sera of individual mice. As shown in FIGS. **7**B and **7**C (right panels), circulating λ light chain concentrations were significantly lower in mice treated with humanized 1F6 as compared to

untreated mice. Mean serum levels of λ light chain in L363-bearing mice treated with humanized 1F6 were 0.006 μg/mL compared to 0.10 μg/mL in sera of untreated mice. Similarly, λ light chain levels in humanized 1F6-treated MM.1S-bearing mice were 0.03 μg/mL compared to 1.25 μg/mL in untreated mice. These results were consistent with the increased survival rates of the mice (FIGS. **7**B and **7**C, right panels).

Example 9: In Vitro Deletion of CD70$^+$ Antigen-Specific T Cells by Humanized 1F6 Antibody

[0238] To test the ability of humanized 1F6 antibody to deplete antigen-specific activated T cells, PBMC from a normal donor expressing HLA-A 0201 were stimulated with the M1 peptide in the presence or absence of varying concentrations of humanized anti-CD70 antibody. Humanized 1F6 antibody (HJLA) was prepared as described above. PBMC were seeded in a 24-well plate at a concentration of 0.5×10^6 cells/ml with 5 μg/ml M1 peptide in 2 ml of medium supplemented with IL-2 and IL-15. On day 5, half of the culture supernatant was replaced with fresh cytokine-containing medium. On day 9, the percentage of antigen-reactive cells (the CD8$^+$/Vβ17$^+$ population) was determined by flow cytometric analysis of cells stained with FITC-conjugated anti-Vβ17- and PE-CyS-conjugated anti-CD8 antibodies.

[0239] FIG. **8**A shows that antigen-specific CD8$^+$/Vβ17$^+$ cells expanded to comprise 33% of all viable cells within the culture in the absence of antibody. In contrast, addition of humanized 1F6 to the cultures on day 0 significantly limited expansion of the antigen-reactive population in an antibody-dose dependent manner. These results show that humanized 1F6 selectively targets and prevents the expansion of antigen-activated T cells.

[0240] In a second study (FIG. **8**B), M1-peptide stimulated cultures were untreated or treated with humanized 1F6 in the absence or presence of antibody that specifically blocks FcγRIII (CD16). In untreated cultures, the antigen-specific CD8+Vβ17$^+$ population expanded to comprise 39% of all viable cells within the culture. Addition of humanized 1F6 significantly diminished expansion of the reactive population. This activity was largely reversed when FcγRIII receptors were blocked with anti-CD16 specific antibody, indicating that deletion of peptide-reactive cells was mediated via humanized 1F6 interaction with FcgRIII-bearing effector cells.

Example 10: Anti-CD70 Antibody does not Affect Antigen-Negative Bystander Cells

[0241] To determine the effect of 1F6-mediated depletion on antigen-negative bystander T cells, the TCR Vβ family representation of CD4 and CD8 lymphocytes was examined in M1-activated cultures that were untreated or treated with a chimeric variant of 1F6 (c1F6) (human IgG1 isotype) and compared to resting, non-antigen stimulated PBMC. Chimeric and humanized 1F6 variants are comparable in binding affinity, capacity to mediate effector functions, and ability to deplete activated CD8+ T cell subsets.

[0242] As shown in FIG. **9**, stimulation of HLA-A 0201$^+$ PBMC with M1 peptide caused the expansion of CD8$^+$ cells bearing the Vβ17 TCR approximately 30-fold, whereas all other Vβ TCR families tested in CD8$^+$ cells and all families tested in the CD4 cell population demonstrated minimal change. In the control population, cell expansion was limited to the Vβ17$^+$CD8$^+$ T cell subset, which increased from <1% of CD8$^+$ cells to 27%; this observation confirms the specificity of the M1-peptide immune response. Unlike T cells stimulated in the absence of CD 70-specific antibody, expansion of M1-peptide specific CD8$^+$ cells was prevented by the addition of c1F6 antibody to the culture. In the presence of c1F6 antibody, the percent Vβ17$^+$CD8$^+$ cells was comparable to that of resting, non-peptide stimulated cells. Treatment with c1F6 antibody did not significantly perturb the relative representations of other CD8$^+$ or CD4$^+$ Vβ TCR families; no group was observed to be eliminated. These data demonstrate that exposure to c1F6 antibody selectively depletes CD70$^+$ activated T cells without causing detectable collateral damage to bystander T cell populations.

Example 11: Mouse Xenograft Model of Renal Cell Carcinoma

[0243] A 786-O subcutaneous xenograft model was used to evaluate antitumor activity of anti-CD70 ADCs administered at different dosages and schedules. Subcutaneous 786-O tumors were initiated in nude mice by implanting tumor fragments (N=5 or 6/group) of approximated 30 mm^3. Tumor growth was allowed to establish and treatment began when average tumor size was approximately 100 mm^3. Tumor dimensions were determined by caliper measurements to monitor growth. Tumor size was calculated using the formula of (length×width^2)/2. In the absence of any treatment, mean tumor volume increased to approximately 600 mm^3 within 40 to 50 days after tumor implantation (see FIG. **10**A). A dose-dependent effect in tumor growth suppression was observed in mice which received either humanized 1F6-mcMMAF4 (HJLA with a loading level of an average of four drug units per antibody) or humanized 1F6-vcMMAF4 (HJLA with a loading level of an average of four drug units per antibody). Detectable delay in tumor growth was observed even at 0.5 and 0.17 mg/kg of h1F6-mcMMAF4 and h1F6-vcMMAF4, respectively.

[0244] Tumor growth was also assessed by time needed for tumors to quadruple in size (see FIG. **10**B). Treatment with either h1F6-mcMMAF4 or h1F6-vcMMAF4 at 0.17 mg/kg significantly delayed the growth of tumors. This delay was observed when the ADCs were given on a q4d×4 or q4d×10 schedule. However, additional administrations as exemplified by the q4d×10 schedule appeared to have a stronger growth inhibitory activity compared to the q4d×4 schedule.

Example 12: Expression of CD70 on Multiple Myeloma Cell Lines

[0245] Cell surface CD70 expression was evaluated in a panel of multiple myeloma cell lines (Table 6). Copy number of CD70 molecules expressed by each cell line was determined by quantitative flow cytometry using the QIFIKit® (Dako, Carpinteria, Calif.). Response of these cells to anti-CD70 ADC-mediated cytotoxicity was determined. In this model, the activity of chimeric anti-CD70 ADCs is a proxy for activity of human anti-CD70 ADCs. Both chimeric 1F6(c1F6)-vcMMAF4 and c1F6-mcMMAF4 were cytotoxic against CD70-expressing multiple myeloma cells. The IC_{50} values obtained with c1F6-vcMMAF4

ranged from 1.2-160 ng/mL while that obtained with c1F6-mcMMAF4 ranged from 1.7-500 ng/mL.

TABLE 6

Cytotoxic Activity of Chimeric Anti-CD70 ADCs against Multiple Myeloma Cell Lines

	CD70	IC_{50} (ng/mL)	
	copies/cell	c1F6-vcMMAF4	c1F6-mcMMAF4
MM.1S	14,000	20	22
MM.1R	25,000	13	20
AMO-1	92,000	16	38
JJN-3	19,000	46	61
L363	13,000	78	210
LB	45,000	80	500
U266	155,000	1.2	1.7
LP-1	34,000	160	155
MOLP-8	9,000	73	33

Example 13: Mouse Xenograft Models of Multiple Myeloma

[0246] The in vivo activity of anti-CD70 ADCs in xenograft models of multiple myeloma was further examined. Human multiple myeloma cell lines MM-1S (FIGS. **11**A & **11**B) or L363 (FIGS. **12**A & **12**B) were resuspended in RPMI-1640 medium at the concentration of 10×10^6 cells/300 μL. To establish tumors 300 μL of cell suspension were injected intravenously through the tail veins of SCID mice. In the MM-1S model, untreated mice succumbed to the injected tumor cells and manifested symptoms around 40 days post tumor implant including hind limb paralysis, hunched posture, cranial swelling, and/or scruffy coat. Mice were euthanized when they demonstrated one or more of these symptoms. Both h1F6(HJLA)-vcMMAF4 and h1F6 (HJLA)-mcMMAF4 provided significant survival benefits to tumor bearing mice compared to control non-binding IgG-vcMMAF4 and IgG-mcMMAF4 (see FIG. **11**A). Tumor burden in the MM-1S model was also assessed by enumerating the number of bone marrow cells expressing human CD138, a plasma cell marker expressed by the MM-1S cells. Bone marrow cells were recovered from mice that were euthanized due to manifestation of symptom or at the end of the experiment on day 122, and the number of CD138-expressing MM-1S cells was determined by flow cytometry. Compared to untreated mice, both control IgG-vcMMAF4 and IgG-mcMMAF4 did not significantly reduce the number of CD138-expressing cells in the bone marrow. On the other hand, h1F6-vcMMAF4 and h1F6-mcMMAF4 significantly reduce tumor burden as demonstrated by much lower number of bone marrow CD138-expressing cells compared to the control ADCs (see FIG. **11**B).

[0247] In the L363 model, disseminated tumor masses develop at multiple locations in mice receiving no treatment, and tumor masses became palpable around 40 days after tumor injection, at which tumor bearing mice would be euthanized. Similar to the MM-1S model, control IgG-vcMMAF4 provided no survival advantage, whereas h1F6-vcMMAF4 significantly prolonged survival (see FIG. **12**A). Since L363 cells secrete immunoglobulin lambda light chain (λ LC), tumor burden can be determined by monitoring the level of human λ LC in the plasma of tumor bearing mice. An ELISA was used to detect secreted λ LC. Ninety six-well flat-bottom Immuno plates (Nunc Maxisorp, #442404, Nalge Nunc International, Rochester, N.Y.) was coated with 1004/well of goat anti-human Ig (Southern Biotech #2010-01, Birmingham, Ala.) at 2 μg/mL in 0.1M sodium carbonate/bicarbonate overnight at 4° C. Wells were washed 5× with 1×PBST (PBS, 0.05% Tween-20), and blocked with 200 4/well of 1% BSA/PBST (0.05% Tween-20) for 1 hour at room temperature. After 5 washes with 1×PBST, serially diluted human λ LC-containing mouse serum samples were added. Purified human λ LC (Bethyl labs, #P80-127, Montgomery, Tex.) was used as the standard. After one hour of incubation at room temperature, wells were washed 5 times with 1×PBST. HRP-goat anti-human lambda chain specific F(ab')2 (Southern Biotech #2072-05) at 1:4000 dilution in 1% BSA/PBST was added. After an additional one hour incubation at room temperature, wells were washed 5 times with 1×PBST. TMB substrate 100 4/well (Sigma, #T8665, St. Louis, Mo.) was used to detect captured λ LC. FIG. **12**B shows the results at forty days after L363 cell implant serum. λ LC levels were comparable between the untreated mice and the IgG-vcMMAF4-treated mice. In contrast, serum λ LC levels in the h1F6-vcMMAF4-treated mice were significantly lower, confirming the ability of anti-CD70 ADC to reduce tumor burden in mice bearing multiple myeloma xenografts.

Example 14: Expression of CD70 on Hodgkin's and Glioblastoma Cell Lines

[0248] Cell surface CD70 expression was also evaluated in panels of Hodgkin's disease (Table 7) and glioblastoma cell lines (Table 8). The copy number of CD70 molecules expressed by each cell line was determined by quantitative flow cytometry using the QIFIKit® (Dako, Carpinteria, Calif.). The response of these cells to chimeric anti-CD70 ADC-mediated cytotoxicity was determined. In this model, the activity of chimeric anti-CD70 ADCs is a proxy for activity of human anti-CD70 ADCs. Both chimeric 1F6 (c1F6)-vcMMAF4 and c1F6-mcMMAF4 were cytotoxic against these CD70-expressing cell lines. In the Hodgkin's disease panel, the IC_{50} values obtained with c1F6-vcMMAF4 ranged from 0.41-42 ng/mL while that obtained with c1F6-mcMMAF4 ranged from 5.2-310 ng/mL (Table 7). In the glioblastoma panel, the IC_{50} values obtained with h1F6-vcMMAF4 ranged from 2.3-27 ng/mL while that obtained with h1F6-mcMMAF4 ranged from 15-110 ng/mL (Table 8).

TABLE 7

Cytotoxic Activity of Anti-CD70 ADCs against Hodgkin's Disease Cell Lines

	CD70	IC_{50} (ng/mL)	
	copies/cell	c1F6-vcMMAF4	c1F6-mcMMAF4
RPMI-6666	21,000	42	230
Hs445	64,000	7.3	310
L428	105,000	1.4	35
KMH2	160,000	0.41	5.2
SUP-HD-1	221,000	6.3	53

TABLE 8

Cytotoxic Activity of Chimeric Anti-CD70 ADCs against Glioblastoma Cell Lines

	CD70	IC_{50} (ng/mL)	
	copies/cell	h1F6-vcMMAF4	h1F6-mcMMAF4
U251	117,000	5.3	15
SNB-19	90,000	12	27
U373MG	70,000	16	35
GMS-10	64,000	27	110
DBTRG-05MG	59,000	2.3	20

[0249] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

[0250] Various references, including patent applications, patents, and scientific publications, are cited herein, the disclosures of each of which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 33

<210> SEQ ID NO 1
<211> LENGTH: 411
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 1

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atccagttgg tgcagtctgg acctgaggtg aagaagcctg gagagacagt caagatctcc 120

tgcaaggctt ctgggtatac cttcacaaac tatggaatga actgggtgaa gcaggctcca 180

ggaaagggtt taaagtggat gggctggata aacacctaca ctggagagcc aacatatgct 240

gatgccttca agggacggtt tgccttctct ttggaaacct ctgccagcac tgcctatttg 300

cagatcaaca acctcaaaaa tgaggacacg gctacatatt tctgtgcaag agactacggc 360

gactatggta tggactactg gggtcaagga acctcagtca ccgtctcctc a 411

<210> SEQ ID NO 2
<211> LENGTH: 137
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 2

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15

Ala Gly Ala Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys
20 25 30

Pro Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Asn Tyr Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu
50 55 60

Lys Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala
65 70 75 80

Asp Ala Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser
85 90 95

Thr Ala Tyr Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr
100 105 110

Tyr Phe Cys Ala Arg Asp Tyr Gly Asp Tyr Gly Met Asp Tyr Trp Gly
115 120 125

Gln Gly Thr Ser Val Thr Val Ser Ser
130 135

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<210> SEQ ID NO 3
<211> LENGTH: 1404
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, human FR in HV Domain

<400> SEQUENCE: 3

atggcttggg tgtggacctt gctattcctg atggcagctg cccaaagtgc ccaagcacag 60

gttcagctgg tgcagtctgg agctgaggtg aagaagcctg gggcctcagt gaaggtctcc 120

tgcaaggctt ctggttacac ctttaccaac tatggaatga actgggtgcg acaggcccct 180

ggacaagggc ttgagtggat gggatggatc aacacctaca ctggagagcc aacatatgct 240

gatgccttca agggcagagt caccatgacc acagacacat ccacgagcac agcctacatg 300

gagctgagga gcctgagatc tgacgacacg gccgtgtatt actgtgcgag agactacggc 360

gactatggta tggactactg gggtcaagga accaccgtca ccgtctcctc agctagcacc 420

aagggcccat cggtcttccc cctggcaccc tcctccaaga gcacctctgg gggcacagcg 480

gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca 540

ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac 600

tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc 660

aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt 720

gacaaaactc acacatgccc accgtgccca gcacctgaac tcctgggggg accgtcagtc 780

ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca 840

tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 900

ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 960

cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 1020

tgcaaggtct ccaacaaagc cctcccagcc cccatcgaga aaaccatctc caaagccaaa 1080

gggcagcccc gagaaccaca ggtgtacacc ctgcccccat cccgggatga gctgaccaag 1140

aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag 1200

tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc 1260

gacggctcct tcttcctcta cagcaagctc accgtggaca agagcaggtg gcagcagggg 1320

aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc 1380

ctctccctgt ctccgggtaa atga 1404

<210> SEQ ID NO 4
<211> LENGTH: 467
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, human FR in HV Domain

<400> SEQUENCE: 4

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15

Ala Gln Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe

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```
        35                  40                  45

Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
    50                  55                  60

Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala
65                  70                  75                  80

Asp Ala Phe Lys Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr Ser
                85                  90                  95

Thr Ala Tyr Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val
            100                 105                 110

Tyr Tyr Cys Ala Arg Asp Tyr Gly Asp Tyr Gly Met Asp Tyr Trp Gly
        115                 120                 125

Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
    130                 135                 140

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
145                 150                 155                 160

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
                165                 170                 175

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
            180                 185                 190

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
        195                 200                 205

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
    210                 215                 220

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
225                 230                 235                 240

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445
```

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```
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460

Pro Gly Lys
465
```

<210> SEQ ID NO 5
<211> LENGTH: 354
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, human FR

<400> SEQUENCE: 5

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caggttcagc tggtgcagtc tggagctgag gtgaagaagc ctggggcctc agtgaaggtc     60

tcctgcaagg cttctggtta cacctttacc aactatggaa tgaactgggt gcgacaggcc    120

cctggacaag ggcttgagtg gatgggatgg atcaacacct acactggaga gccaacatat    180

gctgatgcct tcaagggcag agtcaccatg accagagaca catccatcag cacagcctac    240

atggagctga gcaggctgag atctgacgac acggccgtgt attactgtgc gagagactac    300

ggcgactatg gtatggacta ctggggtcaa ggaaccaccg tcaccgtctc ctca          354
```

<210> SEQ ID NO 6
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, human FR

<400> SEQUENCE: 6

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Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Ala Phe
    50                  55                  60

Lys Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asp Tyr Gly Asp Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110

Thr Val Thr Val Ser Ser
        115
```

<210> SEQ ID NO 7
<211> LENGTH: 1404
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, human FR in HV Domain

<400> SEQUENCE: 7

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atggcttggg tgtggacctt gctattcctg atggcagctg cccaaagtgc ccaagcacag     60

gttcagctgg tgcagtctgg agctgaggtg aagaagcctg gggcctcagt gaaggtctcc    120
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tgcaaggctt ctggttacac ctttaccaac tatggaatga actgggtgcg acaggcccct 180

ggacaagggc ttgagtggat gggatggatc aacacctaca ctggagagcc aacatatgct 240

gatgccttca agggcagagt caccatgacc agagacacat ccatcagcac agcctacatg 300

gagctgagca ggctgagatc tgacgacacg gccgtgtatt actgtgcgag agactacggc 360

gactatggta tggactactg gggtcaagga accaccgtca ccgtctcctc agctagcacc 420

aagggcccat cggtcttccc cctggcaccc tcctccaaga gcacctctgg gggcacagcg 480

gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca 540

ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac 600

tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc 660

aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt 720

gacaaaactc acacatgccc accgtgccca gcacctgaac tcctgggggg accgtcagtc 780

ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca 840

tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 900

ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 960

cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 1020

tgcaaggtct ccaacaaagc cctcccagcc cccatcgaga aaaccatctc caaagccaaa 1080

gggcagcccc gagaaccaca ggtgtacacc ctgcccccat cccgggatga gctgaccaag 1140

aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag 1200

tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc 1260

gacggctcct tcttcctcta cagcaagctc accgtggaca agagcaggtg gcagcagggg 1320

aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc 1380

ctctccctgt ctccgggtaa atga 1404

<210> SEQ ID NO 8
<211> LENGTH: 467
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, human FR in HV Domain

<400> SEQUENCE: 8

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15

Ala Gln Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50 55 60

Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala
65 70 75 80

Asp Ala Phe Lys Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser
85 90 95

Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val
100 105 110

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```
Tyr Tyr Cys Ala Arg Asp Tyr Gly Asp Tyr Gly Met Asp Tyr Trp Gly
        115                 120                 125

Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
    130                 135                 140

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
145                 150                 155                 160

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
                165                 170                 175

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
            180                 185                 190

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
        195                 200                 205

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
    210                 215                 220

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
225                 230                 235                 240

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460

Pro Gly Lys
465


<210> SEQ ID NO 9
<211> LENGTH: 354
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, murine residues in human FR
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<400> SEQUENCE: 9

caggttcagc tggtgcagtc tggagctgag gtgaagaagc ctggggcctc agtgaaggtc 60

tcctgcaagg cttctggtta cacctttacc aactatggaa tgaactgggt gcgacaggcc 120

cctggacaag ggcttgagtg gatgggatgg atcaacacct acactggaga gccaacatat 180

gctgatgcct tcaagggcag atttgccttc tctttggaca catccacgag cacagcctac 240

ttgcagatca acagcctgag atctgacgac acggccgtgt attactgtgc gagagactac 300

ggcgactatg gtatggacta ctggggtcaa ggaaccaccg tcaccgtctc ctca 354

<210> SEQ ID NO 10
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, murine residues in human FR

<400> SEQUENCE: 10

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Ala Phe
50 55 60

Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Asn Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Tyr Gly Asp Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser Ser
115

<210> SEQ ID NO 11
<211> LENGTH: 1404
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, murine residues in human FR

<400> SEQUENCE: 11

atggcttggg tgtggacctt gctattcctg atggcagctg cccaaagtgc ccaagcacag 60

gttcagctgg tgcagtctgg agctgaggtg aagaagcctg gggcctcagt gaaggtctcc 120

tgcaaggctt ctggttacac ctttaccaac tatggaatga actgggtgcg acaggcccct 180

ggacaagggc ttgagtggat gggatggatc aacacctaca ctggagagcc aacatatgct 240

gatgccttca agggcagatt tgccttctct ttggacacat ccacgagcac agcctacttg 300

cagatcaaca gcctgagatc tgacgacacg gccgtgtatt actgtgcgag agactacggc 360

gactatggta tggactactg gggtcaagga accaccgtca ccgtctcctc agctagcacc 420

aagggcccat cggtcttccc cctggcaccc tcctccaaga gcacctctgg gggcacagcg 480

gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca 540

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ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac    600

tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc    660

aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt    720

gacaaaactc acacatgccc accgtgccca gcacctgaac tcctgggggg accgtcagtc    780

ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca    840

tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac    900

ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac    960

cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag   1020

tgcaaggtct ccaacaaagc cctcccagcc cccatcgaga aaaccatctc caaagccaaa   1080

gggcagcccc gagaaccaca ggtgtacacc ctgcccccat cccgggatga gctgaccaag   1140

aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag   1200

tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc   1260

gacggctcct tcttcctcta cagcaagctc accgtggaca agagcaggtg gcagcagggg   1320

aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc   1380

ctctccctgt ctccgggtaa atga                                          1404
```

<210> SEQ ID NO 12
<211> LENGTH: 467
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, murine residues in human FR

<400> SEQUENCE: 12

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Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1               5                   10                  15

Ala Gln Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
            20                  25                  30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
        35                  40                  45

Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
    50                  55                  60

Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala
65                  70                  75                  80

Asp Ala Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Thr Ser
                85                  90                  95

Thr Ala Tyr Leu Gln Ile Asn Ser Leu Arg Ser Asp Asp Thr Ala Val
            100                 105                 110

Tyr Tyr Cys Ala Arg Asp Tyr Gly Asp Tyr Gly Met Asp Tyr Trp Gly
        115                 120                 125

Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
    130                 135                 140

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
145                 150                 155                 160

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
                165                 170                 175

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
            180                 185                 190
```

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```
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
        195                 200                 205

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
    210                 215                 220

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
225                 230                 235                 240

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460

Pro Gly Lys
465
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<210> SEQ ID NO 13
<211> LENGTH: 354
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, murine residues in human FR

<400> SEQUENCE: 13

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caggttcagc tggtgcagtc tggagctgag gtgaagaagc ctggggcctc agtgaaggtc     60

tcctgcaagg cttctggtta cacctttacc aactatggaa tgaactgggt gcgacaggcc    120

cctggacaag ggcttaagtg gatgggatgg atcaacacct acactggaga gccaacatat    180

gctgatgcct tcaagggcag agtcaccatg accagagaca catccatcag cacagcctac    240

atggagctga gcaggctgag atctgacgac acggccgtgt attactgtgc gagagactac    300

ggcgactatg gtatggacta ctggggtcaa ggaaccaccg tcaccgtctc ctca          354
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<210> SEQ ID NO 14
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, murine residues in human FR

<400> SEQUENCE: 14

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Lys Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Ala Phe
50 55 60

Lys Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Tyr Gly Asp Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser Ser
115

<210> SEQ ID NO 15
<211> LENGTH: 1404
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, murine residues in human FR

<400> SEQUENCE: 15

atggcttggg tgtggacctt gctattcctg atggcagctg cccaaagtgc ccaagcacag 60

gttcagctgg tgcagtctgg agctgaggtg aagaagcctg gggcctcagt gaaggtctcc 120

tgcaaggctt ctggttacac ctttaccaac tatggaatga actgggtgcg acaggcccct 180

ggacaagggc ttaagtggat gggatggatc aacacctaca ctggagagcc aacatatgct 240

gatgccttca agggcagagt caccatgacc agagacacat ccatcagcac agcctacatg 300

gagctgagca ggctgagatc tgacgacacg gccgtgtatt actgtgcgag agactacggc 360

gactatggta tggactactg gggtcaagga accaccgtca ccgtctcctc agctagcacc 420

aagggcccat cggtcttccc cctggcaccc tcctccaaga gcacctctgg gggcacagcg 480

gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca 540

ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac 600

tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc 660

aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt 720

gacaaaactc acacatgccc accgtgccca gcacctgaac tcctgggggg accgtcagtc 780

ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca 840

tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 900

ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 960

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cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 1020

tgcaaggtct ccaacaaagc cctcccagcc cccatcgaga aaaccatctc caaagccaaa 1080

gggcagcccc gagaaccaca ggtgtacacc ctgcccccat cccgggatga gctgaccaag 1140

aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag 1200

tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc 1260

gacggctcct tcttcctcta cagcaagctc accgtggaca agagcaggtg gcagcagggg 1320

aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc 1380

ctctccctgt ctccgggtaa atga 1404

<210> SEQ ID NO 16
<211> LENGTH: 467
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, murine residues in human FR

<400> SEQUENCE: 16

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15

Ala Gln Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50 55 60

Lys Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala
65 70 75 80

Asp Ala Phe Lys Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser
85 90 95

Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Asp Tyr Gly Asp Tyr Gly Met Asp Tyr Trp Gly
115 120 125

Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
130 135 140

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
145 150 155 160

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
165 170 175

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
180 185 190

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
195 200 205

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
210 215 220

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
225 230 235 240

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
245 250 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met

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            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460

Pro Gly Lys
465


<210> SEQ ID NO 17
<211> LENGTH: 354
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, murine residues in human FR

<400> SEQUENCE: 17

caggttcagc tggtgcagtc tggagctgag gtgaagaagc ctggggcctc agtgaaggtc     60

tcctgcaagg cttctggtta cacctttacc aactatggaa tgaactgggt gcgacaggcc    120

cctggacaag ggcttaagtg gatgggatgg atcaacacct acactggaga gccaacatat    180

gctgatgcct tcaagggcag atttgccttc tctttggaca catccacgag cacagcctac    240

atggagctga ggagcctgag atctgacgac acggccgtgt attactgtgc gagagactac    300

ggcgactatg gtatggacta ctggggtcaa ggaaccaccg tcaccgtctc ctca          354


<210> SEQ ID NO 18
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, murine residues in human FR

<400> SEQUENCE: 18

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15
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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Lys Trp Met
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Ala Phe
    50                  55                  60

Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asp Tyr Gly Asp Tyr Gly Met Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110

Thr Val Thr Val Ser Ser
        115


<210> SEQ ID NO 19
<211> LENGTH: 1404
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, murine residues in human FR

<400> SEQUENCE: 19

atggcttggg tgtggacctt gctattcctg atggcagctg cccaaagtgc ccaagcacag     60

gttcagctgg tgcagtctgg agctgaggtg aagaagcctg gggcctcagt gaaggtctcc    120

tgcaaggctt ctggttacac ctttaccaac tatggaatga actgggtgcg acaggcccct    180

ggacaagggc ttaagtggat gggatggatc aacacctaca ctggagagcc aacatatgct    240

gatgccttca agggcagatt tgccttctct ttggacacat ccacgagcac agcctacatg    300

gagctgagga gcctgagatc tgacgacacg gccgtgtatt actgtgcgag agactacggc    360

gactatggta tggactactg gggtcaagga accaccgtca ccgtctcctc agctagcacc    420

aagggcccat cggtcttccc cctggcaccc tcctccaaga gcacctctgg gggcacagcg    480

gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca    540

ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac    600

tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc    660

aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt    720

gacaaaactc acacatgccc accgtgccca gcacctgaac tcctgggggg accgtcagtc    780

ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca    840

tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac    900

ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac    960

cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag   1020

tgcaaggtct ccaacaaagc cctcccagcc cccatcgaga aaaccatctc caaagccaaa   1080

gggcagcccc gagaaccaca ggtgtacacc ctgcccccat cccgggatga gctgaccaag   1140

aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag   1200

tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc   1260

gacggctcct tcttcctcta cagcaagctc accgtggaca agagcaggtg gcagcagggg   1320

aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc   1380
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ctctccctgt ctccgggtaa atga 1404

<210> SEQ ID NO 20
<211> LENGTH: 467
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, murine residues in human FR

<400> SEQUENCE: 20

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15

Ala Gln Ala Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50 55 60

Lys Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala
65 70 75 80

Asp Ala Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Thr Ser
85 90 95

Thr Ala Tyr Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Asp Tyr Gly Asp Tyr Gly Met Asp Tyr Trp Gly
115 120 125

Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
130 135 140

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
145 150 155 160

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
165 170 175

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
180 185 190

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
195 200 205

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
210 215 220

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
225 230 235 240

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
245 250 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
260 265 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
275 280 285

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
290 295 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
305 310 315 320

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
325 330 335

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Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460

Pro Gly Lys
465
```

<210> SEQ ID NO 21
<211> LENGTH: 396
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 21

```
atggagacag acacactcct gttatgggta ctgctgctct gggttccagg ttccactggt     60

gacattgtgc tgacacagtc tcctgcttcc ttagctgtat ctctggggca gagggccacc    120

atctcatgca gggccagcaa aagtgtcagt acatctggct atagttttat gcactggtat    180

caacagaaac caggacagcc acccaaactc ctcatctatc ttgcatccaa cctagaatct    240

ggggtccctg ccaggttcag tggcagtggg tctgggacag acttcaccct caacatccat    300

cctgtggagg aggaggatgc tgcaacctat tactgtcagc acagtaggga ggttccgtgg    360

acgttcggtg gaggcaccaa gctggaaatc aaacgg                               396
```

<210> SEQ ID NO 22
<211> LENGTH: 132
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 22

```
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala
            20                  25                  30

Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys Ser
        35                  40                  45

Val Ser Thr Ser Gly Tyr Ser Phe Met His Trp Tyr Gln Gln Lys Pro
    50                  55                  60

Gly Gln Pro Pro Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser
65                  70                  75                  80

Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
                85                  90                  95

Leu Asn Ile His Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys
```

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            100                 105                 110

Gln His Ser Arg Glu Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu
        115                 120                 125

Glu Ile Lys Arg
    130


<210> SEQ ID NO 23
<211> LENGTH: 336
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, human FR

<400> SEQUENCE: 23

gacatcgtga tgacccagtc tccagactcc ctggctgtgt ctctgggcga gagggccacc     60

atcaactgca gggccagcaa aagtgtcagt acatctggct atagttttat gcactggtac    120

cagcagaaac caggacagcc tcctaagctg ctcatttacc ttgcatccaa cctagaatcc    180

ggggtccctg accgattcag tggcagcggg tctgggacag atttcactct caccatcagc    240

agcctgcagg ctgaagatgt ggcagtttat tactgtcagc acagtaggga ggttccgtgg    300

acgttcggtc agggcaccaa ggtggaaatc aaacgt                               336


<210> SEQ ID NO 24
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, human FR

<400> SEQUENCE: 24

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1               5                   10                  15

Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Lys Ser Val Ser Thr Ser
            20                  25                  30

Gly Tyr Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
        35                  40                  45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp
    50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65                  70                  75                  80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln His Ser Arg
                85                  90                  95

Glu Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
            100                 105                 110


<210> SEQ ID NO 25
<211> LENGTH: 717
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, human FR

<400> SEQUENCE: 25

atggagacag acacactcct gttatgggta ctgctgctct gggttccagg ttccactggt     60

gacatcgtga tgacccagtc tccagactcc ctggctgtgt ctctgggcga gagggccacc    120

atcaactgca gggccagcaa aagtgtcagt acatctggct atagttttat gcactggtac    180
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cagcagaaac caggacagcc tcctaagctg ctcatttacc ttgcatccaa cctagaatcc 240

ggggtccctg accgattcag tggcagcggg tctgggacag atttcactct caccatcagc 300

agcctgcagg ctgaagatgt ggcagtttat tactgtcagc acagtaggga ggttccgtgg 360

acgttcggtc agggcaccaa ggtggaaatc aaacgtacgg tggctgcacc atctgtcttc 420

atcttcccgc catctgatga gcagttgaaa tctggaactg cctctgttgt gtgcctgctg 480

aataacttct atcccagaga ggccaaagta cagtggaagg tggataacgc cctccaatcg 540

ggtaactccc aggagagtgt cacagagcag gacagcaagg acagcaccta cagcctcagc 600

agcaccctga cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc 660

acccatcagg gcctgagctc gcccgtcaca aagagcttca acaggggaga gtgttag 717

<210> SEQ ID NO 26
<211> LENGTH: 238
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: murine CDR, human FR

<400> SEQUENCE: 26

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala
20 25 30

Val Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Lys Ser
35 40 45

Val Ser Thr Ser Gly Tyr Ser Phe Met His Trp Tyr Gln Gln Lys Pro
50 55 60

Gly Gln Pro Pro Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser
65 70 75 80

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
85 90 95

Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys
100 105 110

Gln His Ser Arg Glu Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val
115 120 125

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
130 135 140

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
145 150 155 160

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
165 170 175

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
180 185 190

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
195 200 205

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
210 215 220

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> SEQ ID NO 27

-continued

<211> LENGTH: 411
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 27

atggaatgga cctgggtctt tctcttcctc ctgccagtaa ctgcagatgt ccaatcccag 60

gttcagctgc aacagtctgg aactgagctg atgacgcctg gggcctcagt gacgatgtcc 120

tgcaagactt ctggctacac attcagtacc tactggatag agtgggtaaa acagaggcct 180

ggacatggcc ttgagtggat tggagaaatt ttacctggaa gtggttatac tgactacaat 240

gagaagttca aggccaaggc cacattcact gcagatacat cctccaacac agcctacatg 300

caactcagca gcctggcatc tgaggactct gccgtctatt actgtgcaag atgggatagg 360

ctctatgcta tggactactg gggtcaagga acctcagtca ccgtctcctc a 411

<210> SEQ ID NO 28
<211> LENGTH: 137
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 28

Met Glu Trp Thr Trp Val Phe Leu Phe Leu Leu Ser Val Thr Ala Asp
1 5 10 15

Val Gln Ser Gln Val Gln Leu Gln Gln Ser Gly Thr Glu Leu Met Thr
20 25 30

Pro Gly Ala Ser Val Thr Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe
35 40 45

Ser Thr Tyr Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu
50 55 60

Glu Trp Ile Gly Glu Ile Leu Gly Pro Ser Gly Tyr Thr Asp Tyr Asn
65 70 75 80

Glu Lys Phe Lys Ala Lys Ala Thr Phe Thr Ala Asp Thr Ser Ser Asn
85 90 95

Thr Ala Tyr Met Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Trp Asp Arg Leu Tyr Ala Met Asp Tyr Trp Gly
115 120 125

Gly Gly Thr Ser Val Thr Val Ser Ser
130 135

<210> SEQ ID NO 29
<211> LENGTH: 396
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 29

atggagacag acacactcct gttatgggta ctgctgctct gggttccagg ttccactggt 60

gacattgtgc tgacacagtc tcctgcttcc ttaactgtat ctctggggca gaagaccacc 120

atctcatgca gggccagcaa gagtgtcagt acatctggct atagttttat gcactggtac 180

caactgaaac caggacagtc acccaaactc ctcatctatc ttgcgtccaa cctaccatct 240

ggggtccctg ccaggttcag tggcagtggg tctgggacag acttcaccct caaaatccat 300

cctgtggagg aggaggatgc tgcaacctat tactgtcagc acagtaggga gattccgtac 360

acgttcggag gggggaccaa gctggaaata acacgg 396

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<210> SEQ ID NO 30
<211> LENGTH: 132
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 30

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Thr
20 25 30

Val Ser Leu Gly Gln Lys Thr Thr Ile Ser Cys Arg Ala Ser Lys Ser
35 40 45

Val Ser Thr Ser Gly Tyr Ser Phe Met His Trp Tyr Gln Leu Lys Pro
50 55 60

Gly Gln Ser Pro Lys Leu Leu Ile Tyr Leu Ala Ser Asp Leu Pro Ser
65 70 75 80

Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
85 90 95

Leu Lys Ile His Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys
100 105 110

Gln His Ser Arg Glu Ile Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu
115 120 125

Glu Ile Thr Arg
130

<210> SEQ ID NO 31
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: human germline VH exon VH1-2 and JH exon JH6

<400> SEQUENCE: 31

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Tyr Tyr Tyr Tyr Tyr Gly Met Asp Val Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser Ser
115

<210> SEQ ID NO 32
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:

-continued

<223> OTHER INFORMATION: human germline VH exon VH1-18 and JH exon JH6

<400> SEQUENCE: 32

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Ser Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Leu
50 55 60

Gln Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Tyr Tyr Tyr Tyr Tyr Gly Met Asp Val Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser Ser
115

<210> SEQ ID NO 33
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: human germline VL exon B3 and JL exon JK-1

<400> SEQUENCE: 33

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30

Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Tyr Tyr Ser Thr Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
100 105 110

Lys

1-38. (canceled)

39. A method for treating diffuse large B-cell lymphomas in a human subject, comprising

administering to a subject having diffuse large B-cell lymphoma an effective amount of a humanized antibody or antigen binding fragment, comprising:

(i) a humanized heavy chain comprising the three CDRs from SEQ ID NO:2 and a variable region framework sequence of human germline V_H1-2 or V_H1-18 and exon J_H-6, provided that any of positions H46, H67, H68, H69, H70, H71, H80, H81, H82, H82A and H91 (Kabat numbering) can be occupied by the amino acid occupying the corresponding position from SEQ ID NO:2; and

(ii) a humanized light chain comprising the three CDRs from SEQ ID NO:22 and a variable region framework sequence of human germline V_χ exon B3 and J_χ exon J_χ-1, provided that any of positions L25 and L33 can be occupied by the amino acid occupying the corresponding position from SEQ ID NO:22.

40. The method of claim **39**, wherein position H46 is occupied by the amino acid occupying the corresponding position from SEQ ID NO:2.

41. The method of claim **39**, wherein at least one of positions H46, H67, H68, H69, H70, H71, H80, H81, H82, H82A and H91 (Kabat numbering) in the humanized heavy chain variable region is occupied by the residue occupying the corresponding position in SEQ ID NO:2.

42. The method of claim **39**, wherein at least one of positions L25 and L33 in the humanized light chain variable region is occupied by the residue occupying the corresponding position in SEQ ID NO:22.

43. The method of claim **39**, wherein (i) the humanized heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:18, or amino acids 20-137 of SEQ ID NO:4, and (ii) the humanized light chain variable region comprises the amino acid sequence of SEQ ID NO:24.

44. The method of claim **39**, wherein at least one of positions H71 and H91 (Kabat numbering) in the humanized heavy chain variable region is occupied by the residue occupying the corresponding position in SEQ ID NO:2.

45. The method of claim **39**, wherein the humanized heavy chain variable region comprises the sequence set forth in SEQ ID NO:14 or SEQ ID NO: 18, and the humanized light chain variable region comprises the sequence set forth in SEQ ID NO:24.

46. The method of claim **39**, wherein the humanized heavy chain comprises the amino acid sequence corresponding to positions 20-467 of SEQ ID NO:16 or positions 20-467 of SEQ ID NO:20.

47. The method of claim **46**, wherein the humanized heavy chain comprises the amino acid sequence corresponding to positions 20-467 of SEQ ID NO:16

48. The method of claim **47**, wherein the humanized light chain comprises the amino acid sequence corresponding to positions 21-238 of SEQ ID NO:26.

49. The method of claim **39**, wherein the humanized antibody or antigen-binding fragment is an antigen-binding fragment.

50. The method of claim **39**, wherein the humanized antibody or antigen binding fragment further comprises an antibody effector domain.

51. The method of claim **50**, wherein the antibody effector domain mediates ADCC, ADCP or CDC.

52. The method of claim **39**, wherein the antibody or antigen-binding fragment is conjugated to a chemotherapeutic agent.

53. The method of claim **52**, wherein the chemotherapeutic agent is an anti-tubulin agent.

54. The method of claim **53**, wherein the anti-tubulin agent is MMAE or MMAF.

55. A polynucleotide encoding a humanized heavy chain variable region comprising the amino acid sequence of SEQ ID NO:14.

56. The polynucleotide of claim **55** comprising the nucleotide sequence of SEQ ID NO:13.

57. A polynucleotide encoding a humanized light chain variable region comprising the amino acid sequence of SEQ ID NO:24.

58. The polynucleotide of claim **57** comprising the nucleotide sequence of SEQ ID NO:23.

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