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GRIBBEN, J.G., et al., "Rediscovering alemtuzumab: current and emerging therapeutic roles," British Journal of Haematology, 2009, Vol. 144, pages 818-831
ISAACS, J.D., et al., "Humanised monoclonal antibody therapy for rheumatoid arthritis," The Lancet, 1992, Vol. 340, pages 748-752

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(57) Abstract: The present invention relates to humanized immunoglobulins, mouse monoclonal antibodies and chimeric antibodies that have binding specificity for human CD52. The present invention further relates to a humanized immunoglobulin light chain and a humanized immunoglobulin heavy chain. The invention also relates to isolated nucleic acids, recombinant vectors and host cells that comprise a sequence which encodes a humanized immunoglobulin or immunoglobulin light chain or heavy chain, and to a method of preparing a humanized immunoglobulin. The humanized immunoglobulins can be used in therapeutic applications to treat, for example, autoimmune disease, cancer, non-Hodgkin's lymphoma, multiple sclerosis and chronic lymphocytic leukemia.



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ANTI-HUMAN CD52 IMMUNOGLOBULINS

[0001] This application claims priority from U.S. Provisional Application 61/177,837, filed May 13, 2009. The disclosure of that application is incorporated by reference herein in its entirety.

5

BACKGROUND OF THE INVENTION

- [0002] CD52 is a glycosylated, glycosylphosphatidylinositol (GPI)-anchored cell surface protein found in abundance (500,000 molecules/cell) on a variety of normal and malignant lymphoid cells (e.g., T and B cells). *See, e.g.,* Hale et al., *J Biol regul Homeost Agents* **15**:386-391 (2001); Huh et al., *Blood* **92**: Abstract 4199 (1998); Elsner et al., *Blood* **88**:4684-4693 (1996); Gilleece et al., *Blood* **82**:807-812 (1993); Rodig et al., *Clin Cancer Res* **12**:7174-7179 (2006); Ginaldi et al., *Leuk Res* **22**:185-191 (1998). CD52 is expressed at lower levels on myeloid cells such as monocytes, macrophages, and dendritic cells, with little expression found on mature natural killer (NK) cells, neutrophils, and hematological stem cells. *Id.* CD52 is also produced by epithelial cells in the epididymis and duct deferens, and is acquired by sperm during passage through the genital tract (Hale et al., 2001, *supra*; Domagala et al., *Med Sci Monit* **7**:325-331 (2001)). The exact biological function of CD52 remains unclear but some evidence suggests that it may be involved in T cell migration and co-stimulation (Rowan et al., *Int Immunol* **7**:69-77 (1995); Masuyama et al., *J Exp Med* **189**:979-989 (1999); Watanabe et al., *Clin Immunol* **120**:247-259 (2006)).
- [0003] Campath-1H[®] (alemtuzumab, Campath[®], MabCampath[®]) is a humanized anti-human CD52 monoclonal antibody that exhibits potent *in vitro* cytotoxic effects (antibody-dependent cell mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC)). Campath[®] recognizes an epitope which consists of the carboxy terminal four amino acids of the mature CD52 protein and a portion of the negatively charged GPI anchor. Due to its significant

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cytotoxic effects, Campath® is capable of depleting CD52 positive cells *in vivo* and it is approved for front line and third line treatment of chronic lymphocytic leukemia (CLL). Campath® has been evaluated for its utility in the treatment of several autoimmune diseases, including rheumatoid arthritis, vasculitis, myositis and Wegener's disease.

- 5 However, the most advanced studies of Campath® are in treating relapsing remitting multiple sclerosis (MS). These studies showed a significant improvement in time to relapse relative to an active comparator (Rebif® (i.e., interferon beta-1a)).

[0004] A need exists for additional therapeutic agents and approaches to target CD52.

- [0004A]** Throughout this specification and the claims which follow, unless the context
10 requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

- [0004B]** The reference in this specification to any prior publication (or information
15 derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

20 SUMMARY OF THE INVENTION

Humanized Immunoglobulins

- [0005]** The invention relates to humanized immunoglobulins that have binding
specificity for human CD52 (huCD52). They may comprise the complementarity
determining regions (CDRs) of mouse anti-human CD52 antibodies. The humanized
25 immunoglobulins of the invention have amino acid sequences that are different from other humanized immunoglobulins, and in particular from other humanized immunoglobulins that comprise CDRs of murine anti-human CD52 antibodies. The humanized immunoglobulins of the invention are different from the humanized immunoglobulin Campath®. In some embodiments, they provide advantages over humanized antibodies
30 that comprise the CDRs of Campath®.

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[0006] The humanized immunoglobulins described herein can comprise a humanized heavy chain and a humanized light chain. In one embodiment, the humanized immunoglobulin comprises a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 3 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 16; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 4 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 17; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 5 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 18; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 6 and a heavy chain comprising one or more CDRs of SEQ ID NO: 19; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 7 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 20; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 8 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 21; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 9 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 22; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 10 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 23; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 11 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 24; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 12 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 25; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 12 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 137; or a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 13 and a heavy chain sequence comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 26. The CDRs in the above-mentioned SEQ ID NOs are indicated by FIGS. 2 and 3 and are referred to in Tables 1-6 as provided herein.

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[0006A] In another aspect of the invention there is provided a monoclonal anti-human CD52 antibody or an antigen-binding portion that binds to human CD52, wherein the light chain and heavy chain of said antibody comprise the three complementarity determining regions (CDRs) found in

- 5 a) SEQ ID NOs: 3 and 16, respectively;
- b) SEQ ID NOs: 4 and 17, respectively;
- c) SEQ ID NOs: 5 and 18, respectively;
- d) SEQ ID NOs: 6 and 19, respectively;
- e) SEQ ID NOs: 7 and 20, respectively;
- 10 f) SEQ ID NOs: 8 and 21, respectively;
- g) SEQ ID NOs: 9 and 22, respectively;
- h) SEQ ID NOs: 10 and 23, respectively;
- i) SEQ ID NOs: 11 and 24, respectively;
- j) SEQ ID NOs: 12 and 25, respectively;
- 15 k) SEQ ID NOs: 12 and 137, respectively; or
- l) SEQ ID NOs: 13 and 26, respectively.

[0006B] In another aspect of the invention there is provided a monoclonal anti-human CD52 antibody or an antigen-binding portion that binds to human CD52, wherein the heavy chain and the light chain of said antibody comprise the amino acid sequences of

20 SEQ ID NOs: 279 and 280, respectively, without the signal sequences.

[0006C] In another aspect of the invention there is provided a monoclonal anti-human CD52 antibody or an antigen-binding portion that binds to human CD52, wherein the light chain of said antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 102, 138, 145-148, 153-157, and 164-168.

25 **[0006D]** In another aspect of the invention there is provided a monoclonal anti-human CD52 antibody or an antigen-binding portion that binds to human CD52, wherein the light chain of said antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 273, 275, 278, 280, and 282, without the signal sequences.

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[0006E] In another aspect of the invention there is provided an antibody light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 102, 138, 145-148, 153-157, 164-168, 273, 275, 278, 280, and 282, without the signal sequences if present.

- 5 **[0006F]** In another aspect of the invention there is provided a monoclonal anti-human CD52 antibody or an antigen-binding portion that binds to human CD52, wherein the heavy chain of said antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 103, 136, 137, 139-144, 149-152, and 158-163.

- [0006G]** In another aspect of the invention there is provided a monoclonal anti-human
10 CD52 antibody or an antigen-binding portion that binds to human CD52, wherein the heavy chain of said antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 272, 274, 276, 277, 279, and 281, without the signal sequences.

- [0006H]** In another aspect of the invention there is provided an antibody heavy chain
15 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 103, 136, 137, 139-144, 149-152, 158-163, 272, 274, 276, 277, 279, and 281, without the signal sequences if present.

- [0006I]** In another aspect of the invention there is provided a monoclonal anti-human CD52 antibody or an antigen-binding portion that binds to human CD52, wherein the
20 heavy chain and the light chain of said antibody comprise the amino acid sequences of SEQ ID NOs: 272 and 273, respectively, without the signal sequences.

- [0006J]** In another aspect of the invention there is provided a monoclonal anti-human CD52 antibody or an antigen-binding portion that binds to human CD52, wherein the heavy chain and the light chain of said antibody comprise the amino acid sequences of
25 SEQ ID NOs: 274 and 275, respectively, without the signal sequences.

[0006K] In another aspect of the invention there is provided a monoclonal anti-human CD52 antibody or an antigen-binding portion that binds to human CD52, wherein the heavy chain and the light chain of said antibody comprise the amino acid sequences of SEQ ID NOs: 276 and 278, respectively, without the signal sequences.

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[0006L] In another aspect of the invention there is provided a monoclonal anti-human CD52 antibody or an antigen-binding portion that binds to human CD52, wherein the heavy chain and the light chain of said antibody comprise the amino acid sequences of SEQ ID NOs: 277 and 278, respectively, without the signal sequences.

- 5 **[0006M]** In another aspect of the invention there is provided a monoclonal anti-human CD52 antibody or an antigen-binding portion that binds to human CD52, wherein the heavy chain and the light chain of said antibody comprise the amino acid sequences of SEQ ID NOs: 281 and 282, respectively, without the signal sequences.

- [0007]** In another embodiment, the humanized immunoglobulin that has binding
 10 specificity for human CD52 comprises a light chain comprising one or more CDRs (*e.g.*, all three) selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID
 15 NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, and SEQ ID NO: 48; a heavy chain comprising one or more CDRs (*e.g.*, all three) selected from the group consisting of SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63,
 20 SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, and SEQ ID NO: 294; or such a light chain and such a heavy chain; wherein the humanized immunoglobulin is not Campath®.

- [0008]** In another embodiment, the humanized immunoglobulin that has a binding
 25 specificity for human CD52 comprises a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or SEQ ID NO: 13; a heavy

chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, or SEQ ID NO: 137; or such a light chain and such a heavy chain; wherein the humanized immunoglobulin is not Campath®.

- 5 [0009] In some embodiments, the framework region of the humanized immunoglobulin has at least 50% homology to the framework region of the immunoglobulin from which the light chain CDRs and the heavy chain CDRs are obtained. For example, the framework region of the humanized immunoglobulin can be at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or even 100% identical, to a germline human
- 10 immunoglobulin sequence. In one embodiment, the framework region of the humanized immunoglobulin can be obtained or derived from an IgG human antibody variable region. In another embodiment the CD52 is wildtype human CD52. In yet another embodiment, the humanized immunoglobulin can compete with alemtuzumab for binding to human CD52, *e.g.*, it can bind to an epitope that is identical to, or which overlaps with, the epitope to which
- 15 alemtuzumab binds.

- [0010] The invention also relates to a humanized light chain of a humanized immunoglobulin of the invention. In one embodiment, the humanized light chain comprises one or more CDRs selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID
- 20 NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, and SEQ ID NO: 48 or a combination thereof, wherein the humanized light chain is not the humanized light chain of Campath®.

- [0011] In other embodiment, the humanized light chain comprises one or more CDRs (*e.g.*, all
- 25 three CDRs) of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 or SEQ ID NO: 13, wherein the humanized light chain is not the humanized light chain of Campath®.

- [0012] The invention also relates to a humanized heavy chain of a humanized immunoglobulin of the invention. In one embodiment, the humanized heavy chain comprises one or more CDRs of an Ig variable domain selected from the group consisting of SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID
- 30 NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61,

SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, and SEQ ID NO: 294, or a combination thereof, wherein the humanized heavy chain is not the humanized heavy chain of Campath®.

5 [0013] In other embodiments, the humanized heavy chain comprises one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, or SEQ ID NO: 137, wherein the humanized heavy chain is not the humanized heavy chain of Campath®.

10 [0014] Preferably, the humanized immunoglobulins of the present invention comprise both a humanized light chain of the invention and a humanized heavy chain of the invention.

[0015] In other embodiments, the invention provides a humanized immunoglobulin which binds to the same epitope on human CD52 as, or competes or cross-competes with, a mouse monoclonal antibody comprising a light chain variable region of SEQ ID NO: 3 and a heavy chain variable region of SEQ ID NO: 16; a light chain variable region of SEQ ID NO: 4 and a heavy chain variable region of SEQ ID NO: 17; a light chain variable region of SEQ ID NO: 5 and a heavy chain variable region of SEQ ID NO: 18; a light chain variable region of SEQ ID NO: 6 and a heavy chain variable region of SEQ ID NO: 19; a light chain variable region of SEQ ID NO: 7 and a heavy chain variable region of SEQ ID NO: 20; a light chain variable region of SEQ ID NO: 8 and a heavy chain variable region of SEQ ID NO: 21; a light chain variable region of SEQ ID NO: 9 and a heavy chain variable region of SEQ ID NO: 22; a light chain variable region of SEQ ID NO: 10 and a heavy chain variable region of SEQ ID NO: 23; a light chain variable region of SEQ ID NO: 11 and a heavy chain variable region of SEQ ID NO: 24; a light chain variable region of SEQ ID NO: 12 and a heavy chain variable region of SEQ ID NO: 25; or a light chain variable region of SEQ ID NO: 13 and a heavy chain variable region of SEQ ID NO: 26. In other embodiments, the humanized immunoglobulin binds to an epitope on human CD52 which overlaps with the epitope to which such a mouse monoclonal antibody binds.

[0016] In other embodiments, the invention provides a humanized immunoglobulin which binds to an epitope on human CD52 (*e.g.*, SEQ ID NO: 104) comprising at least residue 1 of the mature human CD52 sequence (where residue 1 is the N-terminus of the mature human CD52 sequence, *i.e.*, the N-terminal glycine [G] residue; see FIG. 4). The humanized immunoglobulin

may bind to an epitope comprising at least residues 1, 3, 4 and 5 of the mature human CD52 sequence (these residues being a glycine [G], an asparagine [N], an aspartate [D], and a threonine [T], respectively). The humanized immunoglobulin may bind to an epitope comprising at least residues 1, 2, 3, 4 and 5 of the mature human CD52 sequence (these residues
5 being a glycine [G], a glutamine [Q], an asparagine [N], an aspartate [D], and a threonine [T], respectively). In other embodiments, the invention provides a humanized immunoglobulin which binds to an epitope on human CD52 comprising at least residues 7, 8 and 9 of the mature human CD52 sequence (these residues being a glutamine [Q], a threonine [T], and a serine [S], respectively). In some embodiments, the epitope comprises at least residues 7 (Q), 8 (T) and 11
10 (P) of the mature human CD52 sequence. In some embodiments, the epitope comprises at least residues 4 (D) and 11 (P) of the mature human CD52 sequence.

[0017] In some embodiments, the invention provides a humanized immunoglobulin, which binds to human CD52, and which comprises a light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 115, SEQ ID NO: 118, and SEQ ID NO: 121
15 (*e.g.*, all three of said CDRs), or a heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 124, SEQ ID NO: 127, and SEQ ID NO: 130 (*e.g.*, all three of said CDRs), or both such light chain and such heavy chain. In other embodiments, the invention provides a humanized immunoglobulin, which binds to human CD52, and which comprises a light chain comprising one or more CDRs selected from the group consisting of SEQ ID
20 NO: 116, SEQ ID NO: 119, and SEQ ID NO: 122 (*e.g.*, all three of said CDRs), or a heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 125, SEQ ID NO: 128, and SEQ ID NO: 131 (*e.g.*, all three of said CDRs), or both such light chain and heavy chain. In still further embodiments, the invention provides a humanized immunoglobulin, which binds to human CD52, and which comprises a light chain comprising
25 one or more CDRs selected from the group consisting of SEQ ID NO: 117, SEQ ID NO: 120, and SEQ ID NO: 123 (*e.g.*, all three of said CDRs), or a heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 126, SEQ ID NO: 129, and SEQ ID NO: 132 (*e.g.*, all three of said CDRs), or both such light chain and such heavy chains..

[0018] In certain embodiments, the humanized immunoglobulin comprises a light chain
30 comprising the CDRs of SEQ ID NO: 115, SEQ ID NO: 118 and SEQ ID NO: 121 and a heavy chain comprising the CDRs of SEQ ID NO: 124, SEQ ID NO: 127 and SEQ ID NO: 130. In other embodiments, the humanized immunoglobulin comprises a light chain comprising the

CDRs of SEQ ID NO: 116, SEQ ID NO: 119 and SEQ ID NO: 122 and a heavy chain comprising the CDRs of SEQ ID NO: 125, SEQ ID NO: 128 and SEQ ID NO: 131. In other embodiments, the humanized immunoglobulin comprises a light chain comprising the CDRs of SEQ ID NO: 117, SEQ ID NO: 120 and SEQ ID NO: 123 and a heavy chain comprising the

5 CDRs of SEQ ID NO: 126, SEQ ID NO: 129 and SEQ ID NO: 132.

[0019] The humanized immunoglobulins of the present invention are different from the humanized immunoglobulin Campath®.

[0020] The amino acid sequences of the above-mentioned SEQ ID NOs: 115-132 are provided below, and are based on the amino acid sequences that are reported in Tables 1-6 as provided

10 elsewhere herein. In these amino acid sequences, "X" stands for any amino acid, and the symbol "/" indicates that either (or any) of the amino acids depicted adjacent that symbol may be present at the indicated position (*e.g.*, K/R indicates that a lysine or arginine residue is present at the indicated position and F/L/V indicates that a phenylalanine, leucine or valine residue is present at the indicated position).

15 Light Chain CDR-1 Sequences

K/RSSQSLL/V/IXS/TN/DGXS/TYLY (SEQ ID NO: 115)

K/RSSQSLL/V/IHS/TNGXS/TYLY (SEQ ID NO: 116)

RSSQSLVHTNGNS/TYLY (SEQ ID NO: 117)

Light Chain CDR-2 Sequences

20 XVSXXXX (SEQ ID NO: 118)

XVSXRXS (SEQ ID NO: 119)

MVSXRFS (SEQ ID NO: 120)

Light Chain CDR-3 Sequences

XQXXH/R/KF/L/V/IXX (SEQ ID NO: 121)

25 SQSXH/R/KF/L/V/IPX (SEQ ID NO: 122)

SQSXHVPF/P (SEQ ID NO: 123)

Heavy Chain CDR-1 Sequences

GFXFXXYW/YMX (SEQ ID NO: 124)

GFTFXXYW/YMX (SEQ ID NO: 125)

GFTFTDYW/YMS (SEQ ID NO: 126)

5 Heavy Chain CDR-2 Sequences

XIRKXBXXYTXYYXSVKG (SEQ ID NO: 127)

XIRKXNXYTTEYXXSVKG (SEQ ID NO: 128)

FIRNKANGYTTEYXXSVKG (SEQ ID NO: 129)

Heavy Chain CDR-3 Sequences

10 TXXXY/F/W (SEQ ID NO: 130)

TRYXY/F/WFDY (SEQ ID NO: 131)

TRYIF/WFDY (SEQ ID NO: 132)

- [0021] The invention also relates to a humanized light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 115, SEQ ID NO: 118, and SEQ ID NO: 121 (e.g., all three of said CDRs); a humanized light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 116, SEQ ID NO: 119, and SEQ ID NO: 122 (e.g., all three of said CDRs); or a humanized light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 117, SEQ ID NO: 120, and SEQ ID NO: 123 (e.g., all three of said CDRs).
- 20 [0022] The invention also relates to a humanized heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 124, SEQ ID NO: 127, and SEQ ID NO: 130 (e.g., all three of said CDRs); a humanized heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 125, SEQ ID NO: 128, and SEQ ID NO: 131 (e.g., all three of said CDRs); or a humanized heavy chain comprising one or more CDRs selected from
- 25 the group consisting of SEQ ID NO: 126, SEQ ID NO: 129, and SEQ ID NO: 132 (e.g., all three of said CDRs).

[0023] The humanized light chains and humanized heavy chains of the present invention are different from the humanized light chain and the humanized heavy chains of the humanized immunoglobulin Campath®.

[0024] In some embodiments of the present invention, the humanized immunoglobulins of the invention (irrespective of the manner in which they might otherwise be defined, *e.g.*, regardless of whether they might also be defined in terms of the sequence of one or more of their CDRs and/or by their cross-reactivity with a mouse monoclonal antibody or another humanized immunoglobulin): (1) exhibit binding to glycosylated and de-glycosylated CD52 with no apparent preference; (2) exhibit binding specific for glycosylated CD52; (3) exhibit binding specific for de-glycosylated CD52; or (4) exhibit binding preferential for de-glycosylated over glycosylated CD52. In certain embodiments, the humanized immunoglobulins of the invention have a greater binding affinity for glycosylated human CD52 than for non-glycosylated or de-glycosylated human CD52. Indeed, in certain embodiments of the present invention, the humanized immunoglobulins of the present invention exhibit binding that is specific for glycosylated human CD52. Binding affinity for non-glycosylated or de-glycosylated human CD52 may be determined with the use of mature human CD52 that has been de-glycosylated using a glycosidase, *e.g.*, using the endoglycosidase PNGase-F. In certain embodiments of the present invention, the humanized immunoglobulins of the invention bind to an epitope on mature human CD52 which comprises its *N*-linked carbohydrate moiety. This carbohydrate moiety is a sialylated, polylactosamine-containing core-fucosylated tetraantennary *N*-linked oligosaccharide (Treumann, A. et al., (1995) *J. Biol. Chem.* 270:6088-6099). This epitope may also comprise at least residue 1 of the mature human CD52 sequence, at least residue 3 of the mature human CD52 sequence, at least residues 1, 3, 4 and 5 of the mature human CD52 sequence, or at least residues 1, 2, 3, 4 and 5 of the mature human CD52 sequence. In some embodiments, the mouse or chimeric antibodies of the present invention may have any of these binding features.

[0025] Isolated nucleic acid molecules that encode a humanized immunoglobulin, humanized light chain or humanized heavy chain of the invention, as defined elsewhere herein, are also provided. In some embodiments, the invention is an (one or more) isolated nucleic acid molecule encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the humanized light chain comprises one or more CDRs (*e.g.*, all three CDRs) of SEQ

ID NO: 3 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 16; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 4 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 17; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 5 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 18; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 6 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 19; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 7 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 20; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 8 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 21; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 9 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 22; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 10 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 23; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 11 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 24; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 12 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 25; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 12 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 137; or a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 13 and a heavy chain sequence comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 26.

[0026] In some embodiments, the invention is one or more isolated nucleic acid molecules encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the humanized immunoglobulin binds to the same epitope on human CD52 as a mouse monoclonal antibody comprising a light chain variable region of SEQ ID NO: 3 and a heavy chain variable region of SEQ ID NO: 16; a light chain variable region of SEQ ID NO: 4 and a heavy chain variable region of SEQ ID NO: 17; a light chain variable region of SEQ ID NO: 5 and a heavy chain variable region of SEQ ID NO: 18; a light chain variable region of SEQ ID NO: 6 and a heavy chain variable region of SEQ ID NO: 19; a light chain variable region of SEQ ID NO: 7

and a heavy chain variable region of SEQ ID NO: 20; a light chain variable region of SEQ ID NO: 8 and a heavy chain variable region of SEQ ID NO: 21; a light chain variable region of SEQ ID NO: 9 and a heavy chain variable region of SEQ ID NO: 22; a light chain variable region of SEQ ID NO: 10 and a heavy chain variable region of SEQ ID NO: 23; a light chain variable region of SEQ ID NO: 11 and a heavy chain variable region of SEQ ID NO: 24; a light chain variable region of SEQ ID NO: 12 and a heavy chain variable region of SEQ ID NO: 25; or a light chain variable region of SEQ ID NO: 13 and a heavy chain variable region of SEQ ID NO: 26. In other embodiments, the invention is one or more isolated nucleic acid molecules encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the humanized immunoglobulin binds to an epitope on human CD52 which overlaps with the epitope to which such a mouse monoclonal antibody binds.

[0027] In other embodiments, the invention is one or more isolated nucleic acid molecules encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the humanized immunoglobulin binds to an epitope comprising at least residue 1 of mature human CD52; the humanized immunoglobulin binds to an epitope comprising at least residues 1, 3, 4 and 5 of mature human CD52; the humanized immunoglobulin binds to an epitope comprising at least residues 1, 2, 3, 4 and 5 of mature human CD52; or the humanized immunoglobulin binds to an epitope comprising at least residues 7, 8 and 9 of mature human CD52. In some embodiments, the epitope comprises at least residues 7, 8 and 11 of the mature human CD52 sequence. In some embodiments, the epitope comprises at least residues 4 and 11 of the mature human CD52 sequence.

[0028] In other embodiments, the invention is one or more isolated nucleic acid molecules encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the humanized immunoglobulin comprises a light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 115, SEQ ID NO: 118, and SEQ ID NO: 121 (*e.g.*, all three of said CDRs), and/or a heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 124, SEQ ID NO: 127, and SEQ ID NO: 130 (*e.g.*, all three of said CDRs); a light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 116, SEQ ID NO: 119, and SEQ ID NO: 122 (*e.g.*, all three of said CDRs), and/or

a heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 125, SEQ ID NO: 128, and SEQ ID NO: 131 (*e.g.*, all three of said CDRs); or a light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 117, SEQ ID NO: 120, and SEQ ID NO: 123 (*e.g.*, all three of said CDRs), and/or a heavy chain comprising
5 one or more CDRs selected from the group consisting of SEQ ID NO: 126, SEQ ID NO: 129, and SEQ ID NO: 132 (*e.g.*, all three of said CDRs).

[0029] In certain embodiments, the invention is one or more isolated nucleic acid molecules encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the
10 humanized immunoglobulin comprises a light chain comprising the CDRs of SEQ ID NO: 115, SEQ ID NO: 118 and SEQ ID NO: 121 and a heavy chain comprising the CDRs of SEQ ID NO: 124, SEQ ID NO: 127 and SEQ ID NO: 130; a light chain comprising the CDRs of SEQ ID NO: 116, SEQ ID NO: 119 and SEQ ID NO: 122 and a heavy chain comprising the CDRs of SEQ ID NO: 125, SEQ ID NO: 128 and SEQ ID NO: 131; or a light chain comprising the CDRs
15 of SEQ ID NO: 117, SEQ ID NO: 120 and SEQ ID NO: 123 and a heavy chain comprising the CDRs of SEQ ID NO: 126, SEQ ID NO: 129 and SEQ ID NO: 132.

[0030] The one or more nucleic acids of the invention do not encode the humanized immunoglobulin Campath®.

[0031] In other embodiments, the invention is one or more isolated nucleic acid molecules
20 encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the humanized immunoglobulin has a greater binding affinity for glycosylated human CD52 than for non-glycosylated or de-glycosylated human CD52, *e.g.*, exhibits binding that is specific for glycosylated human CD52. The humanized immunoglobulin may bind to an epitope on mature
25 human CD52 which comprises its *N*-linked carbohydrate moiety. This epitope may also comprise at least residue 1 of the mature human CD52 sequence, at least residue 3 of the mature human CD52 sequence, at least residues 1, 3, 4 and 5 of the mature human CD52 sequence, or at least residues 1, 2, 3, 4 and 5 of the mature human CD52 sequence.

[0032] In other embodiments, the invention is an isolated nucleic acid molecule encoding a
30 humanized light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9,

SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 or SEQ ID NO: 13, wherein the humanized light chain is not the humanized light chain of Campath®.

[0033] In other embodiments, the invention is an isolated nucleic acid molecule encoding a humanized heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, or SEQ ID NO: 137, wherein the humanized heavy chain is not the humanized heavy chain of Campath®.

[0034] In other embodiments, the invention is an isolated nucleic acid molecule encoding a humanized light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, and SEQ ID NO: 48, or a combination thereof, wherein the humanized light chain is not the humanized light chain of Campath®.

[0035] In other embodiments, the invention is an isolated nucleic acid molecule encoding a humanized heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, and SEQ ID NO: 294, or a combination thereof, wherein the humanized heavy chain is not the humanized heavy chain of Campath®.

[0036] In other embodiments, the invention is an isolated nucleic acid molecule encoding a humanized light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 115, SEQ ID NO: 118, and SEQ ID NO: 121 (*e.g.*, all three of said CDRs); a humanized light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 116, SEQ ID NO: 119, and SEQ ID NO: 122 (*e.g.*, all three of said CDRs); or a humanized light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 117, SEQ ID NO: 120, and SEQ ID NO: 123 (*e.g.*, all three of said CDRs).

[0037] In other embodiments, the invention is an isolated nucleic acid molecule encoding a humanized heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 124, SEQ ID NO: 127, and SEQ ID NO: 130 (*e.g.*, all three of said CDRs); a humanized heavy chain comprising one or more CDRs selected from the group consisting of
5 SEQ ID NO: 125, SEQ ID NO: 128, and SEQ ID NO: 131 (*e.g.*, all three of said CDRs); or a humanized heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 126, SEQ ID NO: 129, and SEQ ID NO: 132 (*e.g.*, all three of said CDRs).

[0038] The invention also relates to recombinant vectors (*e.g.*, expression vectors, including mammalian cell expression vectors) that comprise a nucleic acid encoding a humanized
10 immunoglobulin (*e.g.*, a humanized light chain and a humanized heavy chain), a humanized light chain, or a humanized heavy chain of the invention. In some embodiments, the invention is a recombinant vector comprising a nucleic acid encoding a humanized immunoglobulin that comprises a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 3 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 16; a
15 light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 4 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 17; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 5 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 18; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 6 and a heavy chain
20 comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 19; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 7 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 20; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 8 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 21; a light chain
25 comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 9 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 22; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 10 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 23; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 11 and a heavy chain
30 comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 24; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 12 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 25; a light chain

comprising one or more CDRs (e.g., all three CDRs) of SEQ ID NO: 12 and a heavy chain comprising one or more CDRs (e.g., all three CDRs) of SEQ ID NO: 137; or a light chain comprising one or more CDRs (e.g., all three CDRs) of SEQ ID NO: 13 and a heavy chain comprising one or more CDRs (e.g., all three CDRs) of SEQ ID NO: 26.

5 **[0039]** In other embodiments, the recombinant vector comprises a nucleic acid encoding a humanized light chain, wherein the humanized light chain comprises one or more CDRs selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, and SEQ ID NO: 48, or a combination thereof, wherein the humanized light chain is not the humanized light chain of Campath®.

[0040] In other embodiments, the recombinant vector comprises a nucleic acid encoding a humanized heavy chain, wherein the humanized heavy chain comprises one or more CDRs selected from the group consisting of SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, and SEQ ID NO: 294, or a combination thereof, wherein the humanized light chain is not the humanized light chain of Campath®.

[0041] In some embodiments, the invention provides a recombinant vector comprising a nucleic acid molecule, or a pair of recombinant vectors comprising nucleic acid molecules, encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the humanized immunoglobulin binds to the same epitope on human CD52 as a mouse monoclonal antibody comprising a light chain variable region of SEQ ID NO: 3 and a heavy chain variable region of SEQ ID NO: 16; a light chain variable region of SEQ ID NO: 4 and a heavy chain variable region of SEQ ID NO: 17; a light chain variable region of SEQ ID NO: 5 and a heavy chain variable region of SEQ ID NO: 18; a light chain variable region of SEQ ID NO: 6 and a heavy chain variable region of SEQ ID NO: 19; a light chain variable region of SEQ ID NO: 7 and a heavy chain variable region of SEQ ID NO: 20; a light chain variable region of SEQ ID

NO: 8 and a heavy chain variable region of SEQ ID NO: 21; a light chain variable region of SEQ ID NO: 9 and a heavy chain variable region of SEQ ID NO: 22; a light chain variable region of SEQ ID NO: 10 and a heavy chain variable region of SEQ ID NO: 23; a light chain variable region of SEQ ID NO: 11 and a heavy chain variable region of SEQ ID NO: 24; a light chain variable region of SEQ ID NO: 12 and a heavy chain variable region of SEQ ID NO: 25; or a light chain variable region of SEQ ID NO: 13 and a heavy chain variable region of SEQ ID NO: 26. In other embodiments, the invention provides a recombinant vector comprising a nucleic acid molecule, or a pair of recombinant vectors comprising nucleic acid molecules, encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the humanized immunoglobulin binds to an epitope on human CD52 which overlaps with the epitope to which such a mouse monoclonal antibody binds.

[0042] In other embodiments, the recombinant vector comprises a nucleic acid molecule, or a pair of recombinant vectors comprise nucleic acid molecules, encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the humanized immunoglobulin binds to an epitope comprising at least residue 1 of mature human CD52; binds to an epitope comprising at least residues 1, 3, 4 and 5 of mature human CD52; binds to an epitope comprising at least residues 1, 2, 3, 4 and 5 of mature human CD52; or binds to an epitope comprising at least residues 7, 8 and 9 of mature human CD52. In some embodiments, the epitope comprises at least residues 7, 8 and 11 of the mature human CD52 sequence. In some embodiments, the epitope comprises at least residues 4 and 11 of the mature human CD52 sequence.

[0043] In some embodiments, the recombinant vector comprises a nucleic acid molecule, or a pair of recombinant vectors comprise nucleic acid molecules, encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the humanized immunoglobulin comprises a light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 115, SEQ ID NO: 118, and SEQ ID NO: 121 (*e.g.*, all three of said CDRs), and/or a heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 124, SEQ ID NO: 127, and SEQ ID NO: 130 (*e.g.*, all three of said CDRs); a light chain comprising one or more CDRs selected from the group consisting of SEQ ID

NO: 116, SEQ ID NO: 119, and SEQ ID NO: 122 (*e.g.*, all three of said CDRs), and/or a heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 125, SEQ ID NO: 128, and SEQ ID NO: 131 (*e.g.*, all three of said CDRs); or a light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 117, SEQ ID NO: 120, and SEQ ID NO: 123 (*e.g.*, all three of said CDRs), and/or a heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 126, SEQ ID NO: 129, and SEQ ID NO: 132 (*e.g.*, all three of said CDRs).

[0044] In certain embodiments, the recombinant vector comprises a nucleic acid molecule, or a pair of recombinant vectors comprise nucleic acid molecules, encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the humanized immunoglobulin comprises a light chain comprising the CDRs of SEQ ID NO: 115, SEQ ID NO: 118 and SEQ ID NO: 121 and a heavy chain comprising the CDRs of SEQ ID NO: 124, SEQ ID NO: 127 and SEQ ID NO: 130; a light chain comprising the CDRs of SEQ ID NO: 116, SEQ ID NO: 119 and SEQ ID NO: 122 and a heavy chain comprising the CDRs of SEQ ID NO: 125, SEQ ID NO: 128 and SEQ ID NO: 131; or a light chain comprising the CDRs of SEQ ID NO: 117, SEQ ID NO: 120 and SEQ ID NO: 123 and a heavy chain comprising the CDRs of SEQ ID NO: 126, SEQ ID NO: 129 and SEQ ID NO: 132.

[0045] The one or more nucleic acids in the recombinant vector or vectors of the present invention do not encode the humanized immunoglobulin Campath®.

[0046] In other embodiments, the recombinant vector comprises a nucleic acid molecule, or a pair of recombinant vectors comprise nucleic acid molecules, encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the humanized immunoglobulin has a greater binding affinity for glycosylated human CD52 than for non-glycosylated or de-glycosylated human CD52, *e.g.*, exhibits binding that is specific for glycosylated human CD52. The humanized immunoglobulin may bind to an epitope on mature human CD52 which comprises its *N*-linked carbohydrate moiety. This epitope may also comprise at least residue 1 of the mature human CD52 sequence, at least residue 3 of the mature human CD52 sequence, at least residues 1, 3, 4 and 5 of the mature human CD52 sequence, or at least residues 1, 2, 3, 4 and 5 of the mature human CD52 sequence.

[0047] In other embodiments, the recombinant vector comprises a nucleic acid molecule encoding a humanized light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 or SEQ ID NO: 13, wherein the

5 humanized light chain is not the humanized light chain of Campath®.

[0048] In other embodiments, the recombinant vector comprises a nucleic acid molecule encoding a humanized heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26,

10 or SEQ ID NO: 137, wherein the humanized heavy chain is not the humanized heavy chain of Campath®.

[0049] In other embodiments, the recombinant vector comprises a nucleic acid molecule encoding a humanized light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 115, SEQ ID NO: 118, and SEQ ID NO: 121 (*e.g.*, all three of said CDRs); a humanized light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 116, SEQ ID NO: 119, and SEQ ID NO: 122 (*e.g.*, all three of said CDRs); or a humanized light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 117, SEQ ID NO: 120, and SEQ ID NO: 123 (*e.g.*, all three of said CDRs), wherein the humanized light chain is not the humanized light chain of Campath®.

20 [0050] In other embodiments, the recombinant vector comprises a nucleic acid molecule encoding a humanized heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 124, SEQ ID NO: 127, and SEQ ID NO: 130 (*e.g.*, all three of said CDRs); a humanized heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 125, SEQ ID NO: 128, and SEQ ID NO: 131 (*e.g.*, all three of said CDRs); or a humanized heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 126, SEQ ID NO: 129, and SEQ ID NO: 132 (*e.g.*, all three of said CDRs), wherein the humanized heavy chain is not the humanized heavy chain of Campath®.

25 [0051] In particular embodiments, the recombinant vector of the invention is an expression vector, such as a mammalian cell expression vector. In certain embodiments, the vector is a plasmid or a viral vector (*e.g.*, an adenoviral or AAV vector).

30 [0052] The invention also relates to a host cell that comprises a (one or more) nucleic acid (*e.g.*, recombinant) encoding a humanized immunoglobulin (humanized light chain and

humanized heavy chain), a humanized light chain or a humanized heavy chain of the invention. In some embodiments, the host cell comprises a recombinant vector (*e.g.*, expression vector, including mammalian cell expression vectors) of the invention.

[0053] In a particular embodiment, the host cell comprises a nucleic acid (one or more nucleic acids) encoding a humanized light chain and a humanized heavy chain, wherein the humanized light chain and the humanized heavy chain associate together to form a humanized immunoglobulin that has binding specificity for human CD52 and wherein the humanized immunoglobulin comprises a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 3 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 16; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 4 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 17; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 5 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 18; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 6 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 19; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 7 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 20; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 8 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 21; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 9 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 22; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 10 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 23; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 11 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 24; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 12 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 25; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 12 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 137; or a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 13 and a heavy chain sequence comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 26.

[0054] In some embodiments, the host cell comprises one or more nucleic acid molecules encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the humanized immunoglobulin binds to the same epitope on human CD52 as a mouse monoclonal antibody comprising a light chain variable region of SEQ ID NO: 3 and a heavy chain variable region of SEQ ID NO: 16; a light chain variable region of SEQ ID NO: 4 and a heavy chain variable region of SEQ ID NO: 17; a light chain variable region of SEQ ID NO: 5 and a heavy chain variable region of SEQ ID NO: 18; a light chain variable region of SEQ ID NO: 6 and a heavy chain variable region of SEQ ID NO: 19; a light chain variable region of SEQ ID NO: 7 and a heavy chain variable region of SEQ ID NO: 20; a light chain variable region of SEQ ID NO: 8 and a heavy chain variable region of SEQ ID NO: 21; a light chain variable region of SEQ ID NO: 9 and a heavy chain variable region of SEQ ID NO: 22; a light chain variable region of SEQ ID NO: 10 and a heavy chain variable region of SEQ ID NO: 23; a light chain variable region of SEQ ID NO: 11 and a heavy chain variable region of SEQ ID NO: 24; a light chain variable region of SEQ ID NO: 12 and a heavy chain variable region of SEQ ID NO: 25; or a light chain variable region of SEQ ID NO: 13 and a heavy chain variable region of SEQ ID NO: 26. In other embodiments, the host cell comprises one or more nucleic acid molecules encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the humanized immunoglobulin binds to an epitope on human CD52 which overlaps with the epitope to which such a mouse monoclonal antibody binds.

[0055] In other embodiments, the host cell comprises one or more nucleic acid molecules encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the humanized immunoglobulin binds to an epitope comprising at least residue 1 of mature human CD52; binds to an epitope comprising at least residues 1, 3, 4 and 5 of mature human CD52; binds to an epitope comprising at least residues 1, 2, 3, 4 and 5 of mature human CD52; or binds to an epitope comprising at least residues 7, 8 and 9 of mature human CD52. In some embodiments, the epitope comprises at least residues 7, 8 and 11 of the mature human CD52 sequence. In some embodiments, the epitope comprises at least residues 4 and 11 of the mature human CD52 sequence.

[0056] In some embodiments, the host cell comprises one or more nucleic acid molecules encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the humanized immunoglobulin comprises a light chain comprising one or more CDRs selected
5 from the group consisting of SEQ ID NO: 115, SEQ ID NO: 118, and SEQ ID NO: 121 (*e.g.*, all three of said CDRs), and/or a heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 124, SEQ ID NO: 127, and SEQ ID NO: 130 (*e.g.*, all three of said CDRs); a light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 116, SEQ ID NO: 119, and SEQ ID NO: 122 (*e.g.*, all three of said CDRs), and/or
10 a heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 125, SEQ ID NO: 128, and SEQ ID NO: 131 (*e.g.*, all three of said CDRs); or a light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 117, SEQ ID NO: 120, and SEQ ID NO: 123 (*e.g.*, all three of said CDRs), and/or a heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 126, SEQ ID NO: 129,
15 and SEQ ID NO: 132 (*e.g.*, all three of said CDRs).

[0057] In some embodiments, the host cell comprises one or more nucleic acid molecules encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the humanized immunoglobulin comprises a light chain comprising the CDRs of SEQ ID NO: 115,
20 SEQ ID NO: 118 and SEQ ID NO: 121 and a heavy chain comprising the CDRs of SEQ ID NO: 124, SEQ ID NO: 127 and SEQ ID NO: 130; a light chain comprising the CDRs of SEQ ID NO: 116, SEQ ID NO: 119 and SEQ ID NO: 122 and a heavy chain comprising the CDRs of SEQ ID NO: 125, SEQ ID NO: 128 and SEQ ID NO: 131; or a light chain comprising the CDRs of SEQ ID NO: 117, SEQ ID NO: 120 and SEQ ID NO: 123 and a heavy chain comprising the
25 CDRs of SEQ ID NO: 126, SEQ ID NO: 129 and SEQ ID NO: 132.

[0058] In other embodiments, the host cell comprises one or more nucleic acid molecules encoding a humanized heavy chain and a humanized light chain which associate together to form a humanized immunoglobulin that has binding specificity for human CD52, wherein the humanized immunoglobulin has a greater binding affinity for glycosylated human CD52 than
30 for non-glycosylated or de-glycosylated human CD52, *e.g.*, exhibits binding that is specific for glycosylated human CD52. The humanized immunoglobulin may bind to an epitope on mature human CD52 which comprises its *N*-linked carbohydrate moiety. This epitope may also

comprise at least residue 1 of the mature human CD52 sequence, at least residue 3 of the mature human CD52 sequence, at least residues 1, 3, 4 and 5 of the mature human CD52 sequence, or at least residues 1, 2, 3, 4 and 5 of the mature human CD52 sequence.

[0059] In some embodiments, the host cell comprises a nucleic acid molecule encoding a

5 humanized light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 or SEQ ID NO: 13. The humanized light chain is not the humanized light chain of Campath®.

[0060] In other embodiments, the host cell comprises a nucleic acid molecule encoding a

10 humanized heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, or SEQ ID NO: 137. The humanized heavy chain is not the humanized heavy chain of Campath®.

[0061] In some embodiments, the host cell comprises a nucleic acid encoding a humanized

15 light chain, wherein the humanized light chain comprises one or more CDRs selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, and SEQ ID NO: 48 or a combination thereof, wherein the humanized light chain is not the humanized light chain of Campath®.

[0062] In other embodiments, the host cell comprises a nucleic acid encoding a humanized heavy chain, wherein the humanized heavy chain comprises one or more CDRs selected from the group consisting of SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52,

25 SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, and SEQ ID NO: 294, or a combination thereof, wherein the humanized heavy chain is not the humanized heavy chain of Campath®.

[0063] The invention also relates to a method of preparing a humanized immunoglobulin that has binding specificity for human CD52 comprising maintaining a host cell of the invention

(*e.g.*, a host cell that contains one or more recombinant nucleic acids that encode a humanized immunoglobulin of the invention (*e.g.*, a humanized light chain and a humanized heavy chain of the invention)) under conditions appropriate for expression of a humanized immunoglobulin, whereby humanized immunoglobulin chains are expressed and a humanized immunoglobulin is produced. In some embodiments, the method further comprises purifying or isolating the humanized immunoglobulin. In some embodiments, the method further comprises combining the purified or isolated humanized immunoglobulin with a physiologically acceptable vehicle or carrier to produce a pharmaceutical composition.

[0064] The invention also relates to a method of preparing a humanized light chain that has binding specificity for human CD52 comprising maintaining a host cell of the invention (*e.g.*, a host cell that contains one or more recombinant nucleic acids that encode a humanized light chain of the invention) under conditions appropriate for expression of a humanized light chain, whereby a humanized light chain is expressed and a humanized light chain is produced. In some embodiments, the method further comprises purifying or isolating the humanized light chain.

[0065] The invention also relates to a method of preparing a humanized heavy chain that has binding specificity for human CD52 comprising maintaining a host cell of the invention (*e.g.*, a host cell that contains one or more recombinant nucleic acids that encode a humanized heavy chain of the invention) under conditions appropriate for expression of a humanized heavy chain, whereby a humanized heavy chain is expressed and a humanized heavy chain is produced. In some embodiments, the method further comprises purifying or isolating the humanized heavy chain.

[0066] The invention further relates to a pharmaceutical composition comprising a humanized immunoglobulin of the invention (*e.g.*, comprising a humanized light chain of the invention and/or a humanized heavy chain of the invention) and a physiologically acceptable vehicle or carrier. In some embodiments, the pharmaceutical composition comprises a unit dose composition.

[0067] The invention also relates to a method of producing a hybridoma that secretes a monoclonal antibody that has binding specificity for human CD52 comprising administering lymphocytes of a mouse transgenic for human CD52 to a non-transgenic mouse of the same, or of a similar, strain (*e.g.*, CD1) as the human CD52 transgenic mouse, thereby producing an immunized, non-transgenic mouse. Splenocytes of the immunized, non-transgenic mouse are fused with immortalized cells, thereby producing a hybridoma. The hybridoma is maintained

under conditions in which it will secrete a monoclonal antibody having binding specificity for human CD52. In some embodiments, FACS analysis is used to detect a hybridoma that secretes a monoclonal antibody that has binding specificity for human CD52. In other embodiments, the strain of the transgenic mouse and the strain of the non-transgenic mouse are identical. In certain embodiments, the CD52 is wildtype human CD52. In some embodiments, the CD52 transgenic mouse and the non-transgenic mouse are CD1 mice. In some embodiments, the lymphocytes used for immunization are obtained from the spleen of the human CD52 transgenic mouse. In some embodiments, the immortalized cells are selected from the group consisting of SP2/0 Ag14 cells and NS1 myeloma cells. The invention also relates to a hybridoma produced by the methods of the invention. Optionally, the monoclonal antibody secreted by the hybridoma is collected and can be further purified (e.g., substantially purified, isolated). In other embodiments, the method further comprises determining the nucleotide sequence of the monoclonal antibody secreted by the hybridoma.

[0068] The invention also relates to a method for treating an autoimmune disease (e.g., multiple sclerosis (MS), rheumatoid arthritis (RA) (See e.g., *Nature Reviews Drug Discovery* 6: 75-92 (2007)), vasculitis (See e.g., *Rheumatology* 39:229-237 (2000)), Behcet's disease (BD) (See e.g., *Rheumatology* 42:1539-1544 (2003)), lupus and celiac disease (Vivas, S., et al., *N. Engl. J. Med.*, 354(23):2514-2515 (2006)), vasculitis, psoriasis, myositis, scleroderma, aplastic anemia, and colitis) in a patient in need thereof, comprising administering to the patient an effective amount of a humanized immunoglobulin of the invention.

[0069] In another aspect, an effective amount of a humanized immunoglobulin of the invention can be administered in conjunction with one or more immunosuppressive agents to prepare a patient in need thereof for a solid organ transplant (Agarwal et al., *Transplant Immunol.*, 20:6-11 (2008)) or a CD34+ stem cell transplant (Burt et al., *The Lancet*, published online January 30, 2009).

[0070] The invention also relates to a method for treating cancer in a patient in need thereof, comprising administering to the patient an effective amount of a humanized immunoglobulin of the invention.

[0071] The invention also relates to a method for treating multiple sclerosis in a patient in need thereof, comprising administering to the patient an effective amount of a humanized immunoglobulin of the invention.

[0072] The invention also relates to a method for treating chronic lymphocytic leukemia in a patient in need thereof, comprising administering to the patient an effective amount of a humanized immunoglobulin of the invention.

[0073] The administration of a humanized immunoglobulin of the present invention may
5 comprise the administration of the humanized immunoglobulin *per se* (e.g., in a pharmaceutical composition), the administration of one or more recombinant vectors encoding the humanized immunoglobulin, or the administration of a host cell which comprises one or more nucleic acids (e.g., one or more recombinant vectors) encoding the humanized immunoglobulins and expresses the humanized immunoglobulin.

10 [0074] The invention also relates to a method of diagnosing a disease selected from the group consisting of autoimmune diseases (e.g., multiple sclerosis, lupus, vasculitis), cancer (e.g., leukemias (e.g., chronic lymphocytic leukemia), and lymphomas (e.g., non-Hodgkin's lymphoma)), transplant (e.g., solid organ transplant (e.g., kidney transplant) and stem cell transplant), comprising assaying a patient sample *in vitro* with a humanized immunoglobulin of
15 the invention.

[0075] The invention also relates to a humanized immunoglobulin of the invention (e.g., comprising a humanized light chain of the invention and/or humanized heavy chain of the invention), a recombinant vector of the invention, or a host cell of the invention, for use in medicine, such as for use in therapy and/or diagnosis of a disease such as for use in treating a
20 disease or disorder described herein such as an autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, and lupus), cancer, a lymphocyte hyper-proliferative condition (e.g., T or B cell malignancies including leukemia such as B-cell chronic lymphocytic leukemia and lymphomas such as non-Hodgkin's lymphoma). See, e.g., Lundin, J., *et al.*, *Blood*, 101:4267-4272 (2003); Rodig, S.J., *et al.*, *Clinical Cancer Research*, 12(23):7174-7179 (2006). The
25 invention also relates to the use of a humanized immunoglobulin, humanized light chain or humanized heavy chain of the invention, a recombinant vector of the invention, or a host cell of the invention, for the manufacture of a medicament for treating a disease or disorder described herein (e.g., autoimmune diseases (e.g., multiple sclerosis, lupus, vasculitis), cancer (e.g.,
leukemias (e.g., chronic lymphocytic leukemia), and lymphomas (e.g., non-Hodgkin's lymphoma)), and transplant (e.g., solid organ transplant (e.g., kidney transplant) and stem cell
30 transplant)').

- [0076] The invention further provides humanized anti-human CD52 antibodies comprising human light chain framework regions that utilize a human Vk2-A18b gene in which residues 36 (Y) and 46 (L) (Kabat numbering) have been substituted. In some embodiments, residue 36 is V or L and residue 46 is R. The invention also provides humanized anti-human CD52 antibodies
- 5 comprising human heavy chain framework regions that utilize a human VH 3-23 gene in which residue 47 (W) (Kabat numbering) has been substituted. In some embodiments, residues 47 (W) and 49 (S) (Kabat numbering) both have been substituted. In some embodiments, residue 47 is L and residue 49 is S. In other embodiments, residue 47 is L and residue 49 is A.
- [0077] In some embodiments, a humanized anti-human CD52 antibody of the invention has an
- 10 EC_{50} value as determined in a cell-binding assay such as the assay described in Example 29 that is two-fold lower than the EC_{50} value for Campath-1H® antibody. In various embodiments, the humanized anti-human CD52 antibody has an EC_{50} value of 11 nM or less.
- [0078] In some embodiments, a humanized anti-human CD52 antibody of the invention binds CD52 on cells in the presence of anti-Campath-1H® antibodies from the serum of a human
- 15 patient who has been treated with Campath-1H®. That is, the binding of a humanized anti-human CD52 antibody of the invention to CD52 on cells is not reduced in the presence of such anti-Campath-1H® antibodies compared to Campath-1H® binding to CD52 or is less reduced in the presence of such anti-Campath-1H® antibodies compared to Campath-1H® binding to CD52.
- 20 [0079] The invention further provides humanized anti-human CD52 antibodies with a lymphocyte depletion profile in blood and/or spleen of a humanized anti-human CD52 antibody provided herein.
- [0080] In some embodiments, a humanized anti-human CD52 antibody of the invention increases the circulating level of one or more of TNF α , IL-6 and MCP-1 in the serum of a
- 25 subject.
- [0081] In some embodiments, a humanized anti-human CD52 antibody of the invention reduces lymphocyte levels in a subject for at least 30 days, at least 50 days, at least 60 days, at least 70 days, at least 80 days or for more than 80 days.
- [0082] In some embodiments, a humanized anti-human CD52 antibody of the invention delays
- 30 the onset of disease and/or decreases the severity of disease as measured by clinical score in a mouse EAE model.

[0083] In some embodiments, a humanized anti-human CD52 antibody of the invention is less immunogenic than Campath-1H® in an immunogenicity assay such as the assay described in Example 69 or 70.

Mouse Monoclonal Immunoglobulins

5 [0084] The invention also relates to mouse monoclonal antibodies (mouse monoclonal immunoglobulins) that have binding specificity for human CD52. In one embodiment, the invention relates to a mouse monoclonal antibody that has binding specificity for human CD52, comprising a light chain comprising SEQ ID NO: 3 and a heavy chain comprising SEQ ID NO: 16; a light chain comprising SEQ ID NO: 4 and a heavy chain comprising SEQ ID NO: 17;
10 a light chain comprising SEQ ID NO: 5 and a heavy chain comprising SEQ ID NO: 18; a light chain comprising SEQ ID NO: 6 and a heavy chain comprising SEQ ID NO: 19; a light chain comprising SEQ ID NO: 7 and a heavy chain comprising SEQ ID NO: 20; a light chain comprising SEQ ID NO: 8 and a heavy chain comprising SEQ ID NO: 21; a light chain comprising SEQ ID NO: 9 and a heavy chain comprising SEQ ID NO: 22; a light chain
15 comprising SEQ ID NO: 10 and a heavy chain comprising SEQ ID NO: 23; a light chain comprising SEQ ID NO: 11 and a heavy chain comprising SEQ ID NO: 24; a light chain comprising SEQ ID NO: 12 and a heavy chain comprising SEQ ID NO: 25; or a light chain comprising SEQ ID NO: 13 and a heavy chain comprising SEQ ID NO: 26.

[0085] In one embodiment, the mouse monoclonal antibody that has binding specificity for
20 human CD52 comprises a light chain variable region selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13, or a heavy chain variable region selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID
25 NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26, or both such light chain variable region and such heavy chain variable region.

[0086] The invention also relates to a mouse immunoglobulin light chain comprising the variable region of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 or
30 SEQ ID NO: 13.

[0087] The invention also relates to a mouse immunoglobulin heavy chain comprising the variable region of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID

NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25 or SEQ ID NO: 26.

[0088] Preferably, the mouse monoclonal antibodies of the present invention comprise both a mouse antibody light chain of the invention and a mouse antibody heavy chain of the invention.

5 In some embodiments, the invention provides a mouse monoclonal immunoglobulin which binds to the same epitope on human CD52 as a mouse monoclonal antibody comprising a light chain variable region of SEQ ID NO: 3 and a heavy chain variable region of SEQ ID NO: 16; a light chain variable region of SEQ ID NO: 4 and a heavy chain variable region of SEQ ID NO: 17; a light chain variable region of SEQ ID NO: 5 and a heavy chain variable region of
10 SEQ ID NO: 18; a light chain variable region of SEQ ID NO: 6 and a heavy chain variable region of SEQ ID NO: 19; a light chain variable region of SEQ ID NO: 7 and a heavy chain variable region of SEQ ID NO: 20; a light chain variable region of SEQ ID NO: 8 and a heavy chain variable region of SEQ ID NO: 21; a light chain variable region of SEQ ID NO: 9 and a heavy chain variable region of SEQ ID NO: 22; a light chain variable region of SEQ ID NO: 10
15 and a heavy chain variable region of SEQ ID NO: 23; a light chain variable region of SEQ ID NO: 11 and a heavy chain variable region of SEQ ID NO: 24; a light chain variable region of SEQ ID NO: 12 and a heavy chain variable region of SEQ ID NO: 25; or a light chain variable region of SEQ ID NO: 13 and a heavy chain variable region of SEQ ID NO: 26. In other embodiments, the invention provides a mouse monoclonal immunoglobulin which binds to an
20 epitope on human CD52 which overlaps with the epitope to which such a mouse monoclonal antibody binds.

[0089] In other embodiments, the invention provides a mouse monoclonal immunoglobulin which binds to an epitope on human CD52 comprising at least residue 1 of the mature human CD52 sequence. The mouse monoclonal immunoglobulin may bind to an epitope comprising at
25 least residues 1, 3, 4 and 5 of the mature human CD52 sequence, may bind to an epitope comprising at least residues 1, 2, 3, 4 and 5 of the mature human CD52 sequence, or may bind to an epitope comprising at least residues 7, 8 and 9 of the mature human CD52 sequence. In some embodiments, the epitope comprises at least residues 7, 8 and 11 of the mature human CD52 sequence. In some embodiments, the epitope comprises at least residues 4 and 11 of the mature
30 human CD52 sequence.

[0090] The invention also relates to isolated nucleic acid molecules that encode the mouse monoclonal immunoglobulins, mouse immunoglobulin light chains or mouse immunoglobulin

heavy chains of the invention. In some embodiments, the invention is an isolated nucleic acid molecule encoding a mouse immunoglobulin heavy chain and a mouse immunoglobulin light chain which associate together to form a mouse monoclonal immunoglobulin that has binding specificity for human CD52, wherein the mouse immunoglobulin light chain comprises a
5 variable region selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13, or the mouse immunoglobulin heavy chain comprises a variable region selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22,
10 SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26, or both such light chain and such heavy chain.

[0091] In some embodiments, the isolated nucleic acid encodes a mouse immunoglobulin light chain which comprises a variable region selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9,
15 SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13.

[0092] In other embodiments, the isolated nucleic acid encodes a mouse immunoglobulin heavy chain which comprises a variable region selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26.

[0093] The invention also relates to recombinant vectors (*e.g.*, expression vectors, including mammalian cell expression vectors) that comprise a nucleic acid encoding the mouse monoclonal immunoglobulin (*e.g.*, a mouse immunoglobulin light chain and a mouse immunoglobulin heavy chain), the mouse immunoglobulin light chain, or the mouse immunoglobulin heavy chain of the invention. In some embodiments, the invention is a
25 recombinant vector comprising a nucleic acid, or a pair of recombinant vectors comprising nucleic acids encoding a mouse monoclonal immunoglobulin that comprises a light chain variable region selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13, or a heavy chain variable region selected from the
30 group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID

NO: 25, and SEQ ID NO: 26, or both such light chain variable region and heavy chain variable region.

[0094] In other embodiments, the recombinant vector comprises a nucleic acid encoding a mouse immunoglobulin light chain, wherein the mouse immunoglobulin light chain comprises
5 SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 or SEQ ID NO: 13.

[0095] In other embodiments, the recombinant vector comprises a nucleic acid encoding a mouse immunoglobulin heavy chain, wherein the mouse immunoglobulin heavy chain comprises SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20,
10 SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25 or SEQ ID NO: 26.

[0096] In other embodiments, the recombinant vector comprises a nucleic acid encoding a mouse immunoglobulin light chain and a mouse immunoglobulin heavy chain, wherein the mouse immunoglobulin light chain and mouse immunoglobulin heavy chain associate together
15 to form a mouse monoclonal immunoglobulin that has binding specificity for human CD52. In one embodiment, the mouse immunoglobulin light chain comprises a variable region selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13, and the mouse immunoglobulin heavy chain comprises a variable
20 region selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26.

[0097] In particular embodiments, the recombinant vector of the invention is an expression vector, such as a mammalian cell expression vector. In certain embodiments, the vector is a
25 plasmid or a viral vector (*e.g.*, an adenoviral or AAV vector).

[0098] The invention also relates to a host cell that comprises one or more nucleic acids encoding the mouse monoclonal immunoglobulin (mouse immunoglobulin light chain and mouse immunoglobulin heavy chain), the mouse immunoglobulin light chain or the mouse immunoglobulin heavy chain of the invention. For example, in some embodiments, the host cell
30 comprises a recombinant vector (*e.g.*, expression vector, mammalian cell expression vector) of the invention.

[0099] In some embodiments, the host cell comprises nucleic acid encoding a mouse immunoglobulin light chain and a mouse immunoglobulin heavy chain, wherein the mouse immunoglobulin light chain and the mouse immunoglobulin heavy chain associate together to form a mouse monoclonal immunoglobulin that has binding specificity for human CD52 and wherein the mouse immunoglobulin light chain comprises a variable region selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13, and/or the mouse immunoglobulin heavy chain comprises a variable region selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26, or both.

[0100] In some embodiments, the host cell comprises nucleic acid encoding a mouse immunoglobulin light chain, wherein the mouse immunoglobulin light chain comprises a light chain variable region selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13.

[0101] In some embodiments, the host cell comprises a nucleic acid encoding a mouse immunoglobulin heavy chain, wherein the mouse immunoglobulin heavy chain comprises a heavy chain variable region selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26.

[0102] The invention also relates to a method of preparing a mouse monoclonal immunoglobulin comprising maintaining a host cell of the invention (*e.g.*, a host cell that contains one or more recombinant nucleic acids (*e.g.*, recombinant vectors) that encode a mouse monoclonal immunoglobulin (*e.g.*, a mouse immunoglobulin light chain and a mouse immunoglobulin heavy chain) of the invention) under conditions appropriate for expression of a mouse monoclonal immunoglobulin, whereby mouse monoclonal immunoglobulin chains are expressed and a mouse monoclonal immunoglobulin is produced. In some embodiments, the method further comprises purifying or isolating the mouse monoclonal immunoglobulin.

[0103] The invention also relates to a method of preparing a light chain of a mouse monoclonal immunoglobulin, comprising maintaining a host cell of the invention containing a nucleic acid encoding a mouse immunoglobulin light chain of the invention under conditions

appropriate for expression of said mouse immunoglobulin light chain, whereby a light chain is expressed. In some embodiments, the method further comprises purifying or isolating the light chain.

- [0104] The invention also relates to a method of preparing a heavy chain of a mouse monoclonal immunoglobulin, comprising maintaining a host cell of the invention containing a nucleic acid encoding a mouse immunoglobulin heavy chain of the invention under conditions appropriate for expression of said mouse immunoglobulin heavy chain, whereby a mouse immunoglobulin heavy chain is expressed. In some embodiments, the method further comprises purifying or isolating the mouse immunoglobulin heavy chain.
- [0105] The invention also relates to a method of diagnosing a disease (e.g., autoimmune diseases (e.g., multiple sclerosis, lupus, vasculitis), cancer (e.g., leukemias (e.g., chronic lymphocytic leukemia), and lymphomas (e.g., non-Hodgkin's lymphoma)), and transplant (e.g., solid organ transplant (e.g., kidney transplant) and stem cell transplant)) comprising assaying a patient sample *in vitro*, with the mouse monoclonal immunoglobulin of the invention (e.g., Lundin, J., *et al.*, *Blood*, 101:4267-4272 (2003); Rodig, SJ, *et al.*, *Clin. Cancer res.*, 12(23):7174-717179 (2006)).

Chimeric Immunoglobulins

- [0106] The invention also relates to chimeric immunoglobulins that have binding specificity for human CD52. Such chimeric immunoglobulins may include the variable regions of any of the mouse monoclonal immunoglobulin of the present invention. In one embodiment, the chimeric immunoglobulin of the invention comprises the light chain variable region of SEQ ID NO: 3 and the heavy chain variable region of SEQ ID NO: 16; the light chain variable region of SEQ ID NO: 4 and the heavy chain variable region of SEQ ID NO: 17; the light chain variable region of SEQ ID NO: 5 and the heavy chain variable region of SEQ ID NO: 18; the light chain variable region of SEQ ID NO: 6 and the heavy chain variable region of SEQ ID NO: 19; the light chain variable region of SEQ ID NO: 7 and the heavy chain variable region of SEQ ID NO: 20; the light chain variable region of SEQ ID NO: 8 and the heavy chain variable region of SEQ ID NO: 21; the light chain variable region of SEQ ID NO: 9 and the heavy chain variable region of SEQ ID NO: 22; the light chain variable region of SEQ ID NO: 10 and the heavy chain variable region of SEQ ID NO: 23; the light chain variable region of SEQ ID NO: 11 and the heavy chain variable region of SEQ ID NO: 24; the light chain variable region of SEQ ID

NO: 12 and the heavy chain variable region of SEQ ID NO: 25; or the light chain variable region of SEQ ID NO: 13 and the heavy chain variable region of SEQ ID NO: 26.

[0107] The invention also relates to a chimeric antibody that has binding specificity for human CD52, comprising a light chain variable region sequence selected from the group consisting of:

5 the light chain variable region of SEQ ID NO: 3, the light chain variable region of SEQ ID NO: 4, the light chain variable region of SEQ ID NO: 5, the light chain variable region of SEQ ID NO: 6, the light chain variable region of SEQ ID NO: 7, the light chain variable region of SEQ ID NO: 8, the light chain variable region of SEQ ID NO: 9, the light chain variable region of SEQ ID NO: 10, the light chain variable region of SEQ ID NO: 11, the light chain variable

10 region of SEQ ID NO: 12 and the light chain variable region of SEQ ID NO: 13, and/or a heavy chain variable region sequence selected from the group consisting of: the heavy chain variable region of SEQ ID NO: 16, the heavy chain variable region of SEQ ID NO: 17, the heavy chain variable region of SEQ ID NO: 18, the heavy chain variable region of SEQ ID NO: 19, the heavy chain variable region of SEQ ID NO: 20, the heavy chain variable region of SEQ ID

15 NO: 21, the heavy chain variable region of SEQ ID NO: 22, the heavy chain variable region of SEQ ID NO: 23, the heavy chain variable region of SEQ ID NO: 24, the heavy chain variable region of SEQ ID NO: 25 and the heavy chain variable region of SEQ ID NO: 26.

[0108] The invention also relates to a chimeric light chain comprising a variable region selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID

20 NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13.

[0109] The invention also relates to a chimeric heavy chain comprising a variable region selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID

25 NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26.

[0110] Preferably, the chimeric immunoglobulins of the present invention comprise both a chimeric light chain of the invention and a chimeric heavy chain of the invention.

[0111] In some embodiments, the invention provides a chimeric immunoglobulin which binds to the same epitope on human CD52 as a mouse monoclonal antibody comprising a light chain

30 variable region of SEQ ID NO: 3 and a heavy chain variable region of SEQ ID NO: 16; a light chain variable region of SEQ ID NO: 4 and a heavy chain variable region of SEQ ID NO: 17; a light chain variable region of SEQ ID NO: 5 and a heavy chain variable region of SEQ ID

NO: 18; a light chain variable region of SEQ ID NO: 6 and a heavy chain variable region of SEQ ID NO: 19; a light chain variable region of SEQ ID NO: 7 and a heavy chain variable region of SEQ ID NO: 20; a light chain variable region of SEQ ID NO: 8 and a heavy chain variable region of SEQ ID NO: 21; a light chain variable region of SEQ ID NO: 9 and a heavy chain variable region of SEQ ID NO: 22; a light chain variable region of SEQ ID NO: 10 and a heavy chain variable region of SEQ ID NO: 23; a light chain variable region of SEQ ID NO: 11 and a heavy chain variable region of SEQ ID NO: 24; a light chain variable region of SEQ ID NO: 12 and a heavy chain variable region of SEQ ID NO: 25; or a light chain variable region of SEQ ID NO: 13 and a heavy chain variable region of SEQ ID NO: 26. In other embodiments, the chimeric immunoglobulin binds to an epitope on human CD52 which overlaps with the epitope to which such a mouse monoclonal antibody binds.

[0112] In other embodiments, the invention provides a chimeric immunoglobulin which binds to an epitope on human CD52 comprising at least residue 1 of the mature human CD52 sequence. The chimeric immunoglobulin may bind to an epitope comprising at least residues 1, 3, 4 and 5 of the mature human CD52 sequence, may bind to an epitope comprising at least residues 1, 2, 3, 4 and 5 of the mature human CD52 sequence, or may bind to an epitope on human CD52 comprising at least residues 7, 8 and 9 of the mature human CD52 sequence. In some embodiments, the epitope comprises at least residues 7, 8 and 11 of the mature human CD52 sequence. In some embodiments, the epitope comprises at least residues 4 and 11 of the mature human CD52 sequence.

[0113] The invention also relates to isolated nucleic acid molecules that encode the chimeric immunoglobulins, chimeric light chains or chimeric heavy chains of the invention. In some embodiments, the invention is an isolated nucleic acid molecule (one or more nucleic acid molecules) encoding a chimeric heavy chain and a chimeric light chain which associate together to form a chimeric immunoglobulin that has binding specificity for human CD52, wherein the chimeric light chain comprises a variable region selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13; and/or the chimeric heavy chain comprises a variable region selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26.

[0114] In some embodiments, the invention is an isolated nucleic acid molecule encoding a chimeric light chain that comprises the variable region of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 or SEQ ID NO: 13.

5 [0115] In some embodiments, the invention is an isolated nucleic acid molecule encoding a chimeric heavy chain that comprises the variable region of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25 or SEQ ID NO: 26.

[0116] The invention also relates to recombinant vectors (*e.g.*, expression vectors, mammalian
10 cell expression vectors) that comprise a nucleic acid encoding the chimeric immunoglobulin (chimeric light chain and chimeric heavy chain), the chimeric light chain, or the chimeric heavy chain of the invention. In some embodiments, the invention is a recombinant vector comprising a nucleic acid (or a pair of recombinant vectors comprising nucleic acids) encoding a chimeric immunoglobulin that comprises a light chain variable region selected from the group consisting
15 of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13; or a heavy chain variable region selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26; or both such light
20 chain and heavy chain.

[0117] In other embodiments, the recombinant vector comprises a nucleic acid encoding a chimeric light chain, wherein the chimeric light chain comprises the variable region of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 or SEQ ID NO: 13.

25 [0118] In other embodiments, the recombinant vector comprises a nucleic acid encoding a chimeric heavy chain, wherein the chimeric heavy chain comprises the variable region of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25 or SEQ ID NO: 26.

[0119] In particular embodiments, the recombinant vector of the invention is an expression
30 vector, such as a mammalian cell expression vector. In certain embodiments, the vector is a plasmid or a viral vector (*e.g.*, an adenoviral or AAV vector).

[0120] The invention also relates to a host cell that comprises one or more nucleic acids (*e.g.*, one or more recombinant vectors) encoding the chimeric immunoglobulin (chimeric light chain and chimeric heavy chain), the chimeric light chain or the chimeric heavy chain of the invention. For example, in some embodiments, the host cell comprises a recombinant vector (*e.g.*,

5 expression vector, mammalian cell expression vector) of the invention.

[0121] In some embodiments, the host cell comprises a recombinant nucleic acid (or a pair of recombinant nucleic acids) encoding a chimeric light chain and a chimeric heavy chain, wherein the chimeric light chain and the chimeric heavy chain associate together to form a chimeric immunoglobulin that has binding specificity for human CD52 and wherein the chimeric light
10 chain comprises a variable region selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13; and/or the chimeric heavy chain comprises a variable region selected from the group consisting of the variable region of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21,
15 SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26.

[0122] In some embodiments, the host cell comprises a recombinant nucleic acid encoding a chimeric light chain, wherein the chimeric light chain comprises a light chain variable region selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ
20 ID NO: 12, and SEQ ID NO: 13.

[0123] In some embodiments, the host cell comprises a recombinant nucleic acid encoding a chimeric heavy chain, wherein the chimeric heavy chain comprises a heavy chain variable region selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID
25 NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26.

[0124] The invention also relates to a method of preparing a chimeric immunoglobulin comprising maintaining a host cell of the invention (*e.g.*, a host cell that contains one or more isolated nucleic acids that encode a chimeric immunoglobulin (*e.g.*, a chimeric light chain and a chimeric heavy chain) of the invention) under conditions appropriate for expression of a
30 chimeric immunoglobulin, whereby chimeric immunoglobulin chains are expressed and a chimeric immunoglobulin is produced. In some embodiments, the method further comprises purifying or isolating the chimeric immunoglobulin.

[0125] The invention also relates to a method of preparing a chimeric light chain comprising maintaining a host cell of the invention (e.g., a host cell that contains a nucleic acid encoding a chimeric light chain of the invention) under conditions appropriate for expression of said chimeric light chain, whereby a chimeric light chain is expressed and a chimeric light chain is produced. In some embodiments, the method further comprises purifying or isolating the chimeric light chain.

[0126] The invention also relates to a method of preparing a chimeric heavy chain comprising maintaining a host cell of the invention (e.g., a host cell that contains a nucleic acid encoding a chimeric heavy chain of the invention) under conditions appropriate for expression of said chimeric heavy chain, whereby a chimeric heavy chain is expressed and a chimeric heavy chain is produced. In some embodiments, the method further comprises purifying or isolating the chimeric heavy chain.

[0127] The invention also relates to a method of diagnosing a disease selected from the group consisting of autoimmune diseases (e.g., multiple sclerosis, lupus, vasculitis), cancer (e.g., leukemias (e.g., chronic lymphocytic leukemia), and lymphomas (e.g., non-Hodgkin's lymphoma)), and transplant (e.g., solid organ transplant (e.g., kidney transplant) and stem cell transplant), comprising assaying a patient sample *in vitro*, with the chimeric immunoglobulin of the invention.

[0128] Further embodiments of this invention are described as follows. In one aspect, the invention relates to a monoclonal anti-human CD52 antibody or an antigen-binding portion thereof, wherein the light chain and heavy chain of said antibody comprise the three complementarity determining regions (CDRs) found in: SEQ ID NOs: 3 and 16, respectively; SEQ ID NOs: 4 and 17, respectively; SEQ ID NOs: 5 and 18, respectively; SEQ ID NOs: 6 and 19, respectively; SEQ ID NOs: 7 and 20, respectively; SEQ ID NOs: 8 and 21, respectively; SEQ ID NOs: 9 and 22, respectively; SEQ ID NOs: 10 and 23, respectively; SEQ ID NOs: 11 and 24, respectively; SEQ ID NOs: 12 and 25, respectively; SEQ ID NOs: 12 and 137, respectively; or SEQ ID NOs: 13 and 26, respectively. In some embodiments, the invention relates to an antibody that binds to the same epitope on human CD52 as the above monoclonal antibody or antigen-binding portion. In some embodiments, the invention relates to an antibody that competes with the above monoclonal antibody or antigen-binding portion. In some embodiments, the invention relates to an antibody that cross-competes with the above monoclonal antibody or antigen-binding portion.

[0129] In some embodiments, any of the above antibodies or antigen-binding portions binds to an amino acid sequence comprising SEQ ID NO: 104. In some related embodiments, the binding of said antibody or portion to SEQ ID NO: 104 may be reduced by an alanine substitution at one or more of residues 4, 7, 8, or 11 of SEQ ID NO: 104.

- 5 [0130] In some embodiments, the antibody is a humanized antibody, a mouse antibody, or a chimeric antibody. In certain embodiments, the framework regions of the heavy chain of said antibody utilize a VH3-72 or VH3-23 human germline sequence, and the framework regions of the light chain of said antibody utilize a VK2 A18b human germline sequence.

- [0131] In some embodiments, the invention relates to a monoclonal anti-human CD52
10 antibody or an antigen-binding portion thereof, wherein said antibody comprises heavy chain (H)-CDR1, H-CDR2, H-CDR3, and light chain (L)-CDR1, L-CDR2, and L-CDR3 whose amino acid sequences are SEQ ID NOs: 51, 59, 69, 29, 36, and 43, respectively; SEQ ID NOs: 50, 60, 69, 29, 37, and 43, respectively; SEQ ID NOs: 50, 61, 68, 29, 38, and 43, respectively; SEQ ID NOs: 50, 61, 69, 29, 36, and 43, respectively; SEQ ID NOs: 50, 62, 69, 29, 39, and 43,
15 respectively; SEQ ID NOs: 52, 61, 70, 30, 40, and 43, respectively; SEQ ID NOs: 53, 63, 71, 31, 36, and 44, respectively; SEQ ID NOs: 54, 64, 71, 31, 36, and 45, respectively; SEQ ID NOs: 55, 63, 72, 31, 36, and 46, respectively; SEQ ID NOs: 56, 65, 73, 32, 41, and 47, respectively; SEQ ID NOs: 56, 65, 294, 32, 41, and 47, respectively; or SEQ ID NOs: 56, 66, 74, 33, 41, and 48, respectively.

- 20 [0132] In some embodiments, the invention relates to a monoclonal anti-human CD52 antibody or an antigen-binding portion thereof, wherein the light chain and heavy chain of said antibody comprise the amino acid sequences of SEQ ID NOs: 3 and 16, respectively; SEQ ID NOs: 4 and 17, respectively; SEQ ID NOs: 5 and 18, respectively; SEQ ID NOs: 6 and 19, respectively; SEQ ID NOs: 7 and 20, respectively; SEQ ID NOs: 8 and 21, respectively; SEQ ID
25 NOs: 9 and 22, respectively; SEQ ID NOs: 10 and 23, respectively; SEQ ID NOs: 11 and 24, respectively; SEQ ID NOs: 12 and 25, respectively; or SEQ ID NOs: 13 and 26, respectively.

- [0133] In some embodiments, the invention relates to a monoclonal antibody or antigen-binding portion thereof, wherein the heavy chain and light chain of said antibody comprise the amino acid sequences of SEQ ID NOs: 103 and 102, respectively; SEQ ID NOs: 136 and 138,
30 respectively; SEQ ID NOs: 137 and 138, respectively; SEQ ID NOs: 139 and 147, respectively; SEQ ID NOs: 149 and 155, respectively; SEQ ID NOs: 149 and 156, respectively; SEQ ID NOs: 158 and 165, respectively; SEQ ID NOs: 158 and 166, respectively; SEQ ID NOs: 159 and 165,

respectively; SEQ ID NOs: 159 and 166, respectively; SEQ ID NOs: 161 and 166, respectively; or SEQ ID NOs: 163 and 166, respectively. In some embodiments, the invention relates to an antibody that binds to the same epitope on human CD52 as the above monoclonal antibody or antigen-binding portion. In some embodiments, the invention relates to an antibody that
5 competes with the above monoclonal antibody or antigen-binding portion. In some embodiments, the invention relates to an antibody that cross-competes with the above monoclonal antibody or antigen-binding portion.

[0134] In certain embodiments, the invention relates to a monoclonal humanized anti-human CD52 antibody or an antigen-binding portion thereof, wherein the heavy chain and the light
10 chain of said antibody comprise the amino acid sequences of SEQ ID NOs: 272 and 273, respectively, without the signal sequences. In certain embodiments, the invention relates to a monoclonal anti-human CD52 antibody or an antigen-binding portion thereof, wherein the heavy chain and the light chain of said antibody comprise the amino acid sequences of SEQ ID
15 NOs: 274 and 275, respectively, without the signal sequences. In certain embodiments, the invention relates to a monoclonal anti-human CD52 antibody or an antigen-binding portion thereof, wherein the heavy chain and the light chain of said antibody comprise the amino acid sequences of SEQ ID NOs: 276 and 278, respectively, without the signal sequences. In certain
20 embodiments, the invention relates to a monoclonal anti-human CD52 antibody or an antigen-binding portion thereof, wherein the heavy chain and the light chain of said antibody comprise the amino acid sequences of SEQ ID NOs: 277 and 278, respectively, without the signal sequences. In certain embodiments, the invention relates to a monoclonal anti-human CD52
25 antibody or an antigen-binding portion thereof, wherein the heavy chain and the light chain of said antibody comprise the amino acid sequences of SEQ ID NOs: 279 and 280, respectively, without the signal sequences. In certain embodiments, the invention relates to a monoclonal anti-human CD52 antibody or an antigen-binding portion thereof, wherein the heavy chain and the light chain of said antibody comprise the amino acid sequences of SEQ ID NOs: 281 and 282, respectively, without the signal sequences. The invention also provides antibodies that bind to the same epitope on CD52 as one of these humanized antibodies and antibodies that compete or cross-compete with one of these humanized antibodies. In related embodiments, the
30 invention provides compositions comprising one such humanized antibody and a pharmaceutically acceptable carrier.

[0135] In some embodiments, the invention relates to a monoclonal anti-human CD52 antibody or an antigen-binding portion thereof, wherein the light chain of said antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 102, 138, 145-148, 153-157, and 164-168. In certain embodiments, the invention relates to a monoclonal anti-human CD52 antibody or an antigen-binding portion thereof, wherein the light chain of said antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 273, 275, 278, 280, and 282, without the signal sequences. In certain embodiments, the invention relates to an antibody light chain or a portion thereof, comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 102, 138, 145-148, 153-157, 164-168, 273, 275, 278, 280, and 282, without the signal sequences if present.

[0136] In some embodiments, the invention relates to a monoclonal anti-human CD52 antibody or an antigen-binding portion thereof, wherein the heavy chain of said antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 103, 136, 137, 139-144, 149-152, and 158-163. In certain embodiments, the invention relates to a monoclonal anti-human CD52 antibody or an antigen-binding portion thereof, wherein the heavy chain of said antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 272, 274, 276, 277, 279, and 281, without the signal sequences. In certain embodiments, the invention relates to an antibody heavy chain or a portion thereof, comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 103, 136, 137, 139-144, 149-152, 158-163, 272, 274, 276, 277, 279, and 281, without the signal sequences if present.

[0137] In some embodiments, any of the above antibodies may be an IgG, IgM, IgA, IgD or IgE molecule. In certain embodiments, said IgG is IgG1, IgG2, IgG3, or IgG4.

[0138] In some embodiments, any of the above antigen-binding portions may be a single chain antibody, Fv, Fab, Fab', F(ab')₂, Fd, single chain Fv molecule (scFv), bispecific single chain Fv dimer, diabody, domain-deleted antibody or single domain antibody (dAb).

[0139] The invention also relates to any of the above antibodies or antigen-binding portions, wherein said antibody or antigen-binding portion depletes T or B lymphocytes, or both; preferentially depletes T lymphocytes as compared to B lymphocytes; increases circulating serum levels of TNF-alpha, IL-6, or MCP-1 (e.g., by at least 5%, at least 10%, at least 50%, at least 100% or at least 200%); mediates antibody-dependent cell mediated cytotoxicity (ADCC) of CD52-expressing cells; mediates complement-dependent cytotoxicity (CDC) of CD52-

expressing cells; binds to human CD52 in spite of the presence of neutralizing antibodies to alemtuzumab in a human patient; and/or promotes intracellular signaling in human T and/or B cells (*see, e.g.,* Hederer et al., *International Immunology* 12:505-616 (2000); Watanabe et al., *Clinical Immunology* 120: 247-259 (2006)).

5 [0140] The invention further relates to an isolated nucleic acid encoding the heavy chain or an antigen-binding portion thereof, or the light chain or an antigen-binding portion thereof, of any of the above antibodies. In some embodiments, said isolated nucleic acid comprises a heavy chain nucleotide sequence selected from the group consisting of SEQ ID NOs: 283, 285, 287, 288, 290, and 292, or said nucleotide sequence without the sequence encoding a signal peptide;
10 a light chain nucleotide sequence selected from the group consisting of SEQ ID NOs: 284, 286, 289, 291, and 293, or said nucleotide sequence without the sequence encoding a signal peptide; or both said heavy chain nucleotide sequence and said light chain nucleotide sequence. In certain embodiments, said isolated nucleic acid comprises a heavy chain nucleotide sequence and a light chain nucleotide sequence selected from the group consisting of SEQ ID NO: 283
15 and SEQ ID NO: 284, respectively, both without sequences encoding signal peptides; SEQ ID NO: 285 and SEQ ID NO: 286, respectively, both without sequences encoding signal peptides; SEQ ID NO: 287 and SEQ ID NO: 289, respectively, both without sequences encoding signal peptides; SEQ ID NO: 288 and SEQ ID NO: 289, respectively, both without sequences encoding signal peptides; SEQ ID NO: 290 and SEQ ID NO: 291, respectively, both without sequences
20 encoding signal peptides; and SEQ ID NO: 292 and SEQ ID NO: 293, respectively, both without sequences encoding signal peptides.

[0141] The invention also relates to the use of an isolated nucleic acid comprising a heavy chain nucleotide sequence and an isolated nucleic acid comprising a light chain nucleotide sequence for the manufacture of a medicament for treating a patient in need thereof, wherein
25 said heavy chain nucleotide sequence and light chain nucleotide sequence are selected from the group consisting of SEQ ID NO: 283 and SEQ ID NO: 284, respectively, both without sequences encoding signal peptides; SEQ ID NO: 285 and SEQ ID NO: 286, respectively, both without sequences encoding signal peptides; SEQ ID NO: 287 and SEQ ID NO: 289, respectively, both without sequences encoding signal peptides; SEQ ID NO: 288 and SEQ ID
30 NO: 289, respectively, both without sequences encoding signal peptides; SEQ ID NO: 290 and SEQ ID NO: 291, both respectively, without sequences encoding signal peptides; and SEQ ID NO: 292 and SEQ ID NO: 293, both respectively, without sequences encoding signal peptides.

[0142] The invention also relates to a recombinant vector comprising (1) a nucleic acid sequence encoding the heavy chain or an antigen-binding portion thereof, (2) a nucleic acid sequence encoding the light chain or an antigen-binding portion thereof, or (3) both, of any of the above antibodies. The invention further relates to a host cell comprising a first nucleic acid sequence encoding the heavy chain or an antigen-binding portion thereof of any of the above antibodies, said first nucleic acid sequence operably linked to an expression control element, and a second nucleic acid sequence encoding the light chain or an antigen-binding portion thereof of said antibody, said second nucleic acid sequence operably linked to an expression control element. The invention relates to a method of making an anti-human CD52 antibody or an antigen-binding portion thereof, comprising maintaining said host cell under conditions appropriate for expression of the antibody or portion, and also relates to said method further comprising the step of isolating the antibody or portion.

[0143] The invention relates to a composition comprising the monoclonal antibody or antigen-binding portion according to any one of claims 1-24 and a pharmaceutically acceptable vehicle or carrier.

[0144] In some embodiments, the invention relates to a method for treating a patient in need thereof, comprising administering to the patient an effective amount of any of the above antibodies or antigen-binding portions, or the above composition. In certain embodiments, said patient is receiving a transplantation.

[0145] In some embodiments, the invention relates to a method for treating an autoimmune disease in a patient in need thereof, comprising administering to the patient an effective amount of any of the above antibodies or antigen-binding portions, or the above composition. In certain embodiments, the autoimmune disease is, e.g., multiple sclerosis, rheumatoid arthritis, or systemic lupus erythematosus.

[0146] In some embodiments, the invention relates to a method for treating cancer in a patient in need thereof, comprising administering to the patient an effective amount of any of the above antibodies or antigen-binding portions, or the above composition. In certain embodiments, the cancer is, e.g., a lymphoma such as non-Hodgkin's lymphoma; a leukemia such as B-cell chronic lymphocytic leukemia; T cell malignancy, wherein the antibody or portion preferentially depletes T cells as compared to B cells; or a solid tumor.

[0147] In some embodiments, any of the above methods of treatment further comprising administering to the patient a neutrophil or NK cell stimulatory agent. In certain embodiments,

said agent is G-CSF or GM-CSF. In some embodiments, any of the above methods of treatment further comprises administering to the patient a T regulatory cell stimulatory agent. In certain embodiments, said agent is rapamycin.

5 [0148] In some embodiments, the invention relates to a method for inhibiting angiogenesis in a patient in need thereof, comprising administering an effective amount of any of the above antibodies or antigen-binding portions to the patient. In certain embodiments, the patient has a solid tumor. In certain embodiments, the patient has neovascularization. In certain
embodiments, said neovascularization is in the eye.

[0149] The invention also relates to the use of any of the above antibodies or antigen-binding
10 portions for the manufacture of a medicament for treating an autoimmune disease in a patient in need thereof. Further, the invention relates to the use of any of the above antibodies or antigen-binding portions for the manufacture of a medicament for treating cancer in a patient in need thereof. The invention relates to the use of any of the above antibodies or antigen-binding portions for the manufacture of a medicament for treating a patient in need of a transplantation.
15 The invention relates to the use of any of the above antibodies or antigen-binding portions for the manufacture of a medicament for treating neovascularization in a patient in need thereof.

[0150] The invention also relates to the use of any of the above antibodies or antigen-binding portions as a medicament.

BRIEF DESCRIPTION OF THE DRAWINGS

20 [0151] FIG. 1A-1B is a schematic representation of the development of new anti-CD52 monoclonal antibodies. The general scheme is depicted in FIG. 1A and the names of the mouse anti-human CD52 antibody clones as well as their isotypes in shown in FIG 1B.

[0152] FIG. 2 is an alignment of the amino acid sequences of several mouse anti-human CD52
25 kappa light chain sequences (SEQ ID NOS:1-13). Campath-1G is the rat monoclonal antibody from which the humanized Campath-1H antibody is derived.

[0153] FIG. 3 is an alignment of the amino acid sequences of several mouse anti-human CD52 heavy chain sequences (SEQ ID NOS:14-26).

[0154] FIG. 4 is an alignment of wildtype CD52 and 10 mutant CD52 proteins (SEQ ID NOS: 104-114, from top to bottom).

30 [0155] FIG. 5A illustrates the FACS-based N-terminal binding profile of antibodies 4B10 and 7F11 on cells expressing CD52 alanine scanning mutants.

- [0156] FIG. 5B illustrates the FACS-based middle region binding profile of antibodies CF1D12, 3G7, 9D9, 5F7, 4G7, and 11C11 on cells expressing CD52 alanine scanning mutants.
- [0157] FIG. 5C illustrates the FACS-based binding profile of antibodies Campath-1H® ("Campath 1H"), 2C3, 12G6, and 23E6 on cells expressing CD52 alanine scanning mutants.
- 5 [0158] FIG. 5D depicts immunoblots of CD52 +/- N-linked glycosylation probed with the panel of chimeric monoclonal antibodies. "C1H" stands for Campath-1H®.
- [0159] FIG. 6 is a graph showing the results of a 1.5 hour CDC assay on various chimeric anti-CD52 antibodies screened on CHO-K1 CD52 #67 cells. The results show that chimeric antibodies 4B10 and 7F11 are comparable to or better than Campath-1H® ("Campath 1H").
- 10 [0160] FIG. 7 is a graph showing the results of a 14 hour ADCC assay on various chimeric IgG1 antibodies to CD52 screened on CHO-K1 CD52 #67 cells. The results show that chimeric antibodies 2C3 and 12G6 are comparable to or better than Campath-1H® ("Campath 1-H").
- [0161] FIG. 8A-8C illustrate the comparative binding of various anti-CD52 antibodies and the Campath-1H® ("C-1H") antibody to defined human lymphocyte populations. These figures
- 15 show the hierarchy of the binding ability of the chimeric antibodies screened by FACS assay. Curves to the far right demonstrate the highest binding ability, whereas curves to the left bind with lower affinity.
- [0162] FIGS. 9A-9C are graphs illustrating the level of CD4 T cells (FIG. 9A), CD8 T cells (FIG. 9B) and CD19 B cells (FIG. 9C) in the blood 72 hours after dosing with chimeric
- 20 antibodies 7F11, 8G3, 23E6, 12G6, 4B10, or 5F7, or Campath-1H® ("Cam").
- [0163] FIGS. 10A-10C are graphs illustrating the level of CD4 T cells (FIG. 10A), CD8 T cells (FIG. 10B) and CD19 B cells (FIG. 10C) in the spleen 72 hours after dosing with chimeric antibodies 7F11, 8G3, 23E6, 12G6, 4B10, or 5F7, or Campath-1H® ("Cam").
- [0164] FIGS. 11A-11C are graphs showing the level of CD4 T cells (FIG. 11A), CD8 T cells
- 25 (FIG. 11B) and CD19 B cells (FIG. 11C) in the blood 72 hours after dosing with chimeric antibodies 2C3, 9D9, 4B10, 3G7, or 11C11, or Campath-1H® ("Cam").
- [0165] FIG. 12 is a Kaplan Meier Survival graph illustrating the percent of surviving mice after treatment with 7F11, 4B10, or 12G6 chimeric monoclonal antibodies, or Campath-1H® ("Campath").
- 30 [0166] FIG. 13 is a Kaplan Meier Survival graph illustrating the percent of surviving mice after treatment with 2C3, 8G3, or 23E6 chimeric monoclonal antibodies, or Campath-1H® ("Campath").

- [0167] FIG. 14 is a Kaplan Meier Survival graph illustrating the percent of surviving mice after treatment with 9D9 or 4B10 chimeric monoclonal antibodies, or Campath-1H® (“Campath”).
- [0168] FIG. 15 is a Kaplan Meier Survival graph illustrating the percent of surviving mice after treatment with 2C3 or 11C11 chimeric monoclonal antibodies, or Campath-1H® (“Campath”).
- [0169] FIG. 16 is an alignment of the mouse anti-human CD52 antibody 4B10 heavy chain variable region (SEQ ID NO: 96) sequence with the closest matched human germline sequence (SEQ ID NO: 97) and the humanized heavy chain variable region sequence (SEQ ID NO: 98). Also shown is an alignment of the mouse anti-human CD52 antibody 4B10 light chain variable region (SEQ ID NO: 99) sequence with the closest matched human germline sequence (SEQ ID NO: 100) and the humanized light chain variable region sequence (SEQ ID NO: 101).
- [0170] FIG. 17 shows the humanized 4B10 heavy chain (SEQ ID NO: 103) and light chain (SEQ ID NO: 102) variable region sequences.
- [0171] FIG. 18 is a graph showing that humanized antibody 4B10-H1/K1 (“4B10-Humanized”) and chimeric antibody 4B10 bind equivalently to cells expressing CD52.
- [0172] FIG. 19 is a graph showing that humanized antibody 4B10 -H1/K1 (“4B10-Humanized”) and chimeric antibody 4B10 mediate equivalent ADCC activity on cells expressing CD52.
- [0173] FIG. 20 is a graph showing that humanized antibody 4B10 -H1/K1 (“4B10-Humanized”) and chimeric antibody 4B10 mediate equivalent CDC activity on cells expressing CD52.
- [0174] FIG. 21 is a graph illustrating the pharmacokinetic profile of chimeric anti-CD52 antibodies (12G6, 7F11 and 4B10), Campath-1H® (“Campath”), and humanized anti-CD52 antibody 4B10-H1/K1 (“4B10 humanized (H1/K1)”) in heterozygous huCD52 transgenic mice.
- [0175] FIGS. 22A-22C are graphs showing the level of CD4 T cells (FIG. 22A), CD8 T cells (FIG. 22B) and CD19 B cells (FIG. 22C) in the blood 72 hours after dosing with chimeric antibody 4B10 or humanized antibody 4B10-H1/K1 (“4B10-Hu”) or Campath-1H® (“Campath”).
- [0176] FIG. 23 is a graph showing the summary of the relative binding affinities of the anti-CD52 monoclonal antibodies.

[0177] FIG. 24 shows the humanized 7F11 heavy and light (kappa) chain variable region sequences. Amino acid residues that are back mutated to mouse residues are underlined and the CDRs are shown in boldface.

5 [0178] FIG. 25 is a histogram showing that chimeric and humanized 7F11 antibodies bind equivalently to cells expressing CD52. The X axis represents the fluorescence emitted by the bound anti-CD52 antibody, while the area of each peak represents the total cell population.

[0179] FIG. 26A shows the humanized 2C3 heavy chain variable region sequences. Amino acid residues that are back mutated to mouse residues are underlined and the CDRs are shown in boldface. FIG. 26B shows the humanized 2C3 light (kappa) chain variable region sequences.

10 Amino acid residues that are back mutated to mouse residues are underlined and the CDRs are shown in boldface.

[0180] FIG. 27A is a histogram showing binding of humanized and chimeric 2C3 antibodies to cells expressing CD52. The X axis represents the fluorescence emitted by the bound anti-CD52 antibody, while the area of each peak represents the total cell population. FIG. 27B is a

15 histogram showing that chimeric and a subset of the humanized 2C3 antibodies bind equivalently to cells expressing CD52. The X axis represents the fluorescence emitted by the bound anti-CD52 antibody, while the area of each peak represents the total cell population.

[0181] FIG. 28A shows the humanized 12G6 heavy chain variable region sequences. Amino acid residues that are back mutated to mouse residues are underlined and the CDRs are shown in boldface. FIG. 28B shows the humanized 12G6 light (kappa) chain variable region sequences.

20 Amino acid residues that are back mutated to mouse residues are underlined and the CDRs are shown in boldface.

[0182] FIG. 29 is a histogram showing that chimeric and a subset of the humanized 12G6 antibodies bind equivalently to cells expressing CD52. The X axis represents the fluorescence emitted by the bound anti-CD52 antibody, while the area of each peak represents the total cell population.

[0183] FIG. 30A shows the humanized 9D9 heavy chain variable region sequences. Amino acid residues that are back mutated to mouse residues are underlined and the CDRs are shown in boldface. FIG. 30B shows the humanized 9D9 light (kappa) chain variable region sequences.

30 Amino acid residues that are back mutated to mouse residues are underlined and the CDRs are shown in boldface.

[0184] FIG. 31 is a histogram showing that chimeric and a subset of the humanized 9D9 antibodies bind equivalently to cells expressing CD52. The X axis represents the fluorescence emitted by the bound anti-CD52 antibody, while the area of each peak represents the total cell population.

5 [0185] FIG. 32A shows the binding curves of Campath-1H® ("C1H"), a chimeric 2C3 antibody, and a humanized 2C3-SFD1/K12 antibody to primary human T cells and huCD52 transgenic mouse T cells. FIG. 32B shows the binding curves of Campath-1H® ("C1H"), a chimeric 9D9 antibody, and humanized 9D9 antibodies to primary human T cells and huCD52 transgenic mouse T cells. FIG. 32C shows the binding curves of Campath-1H® ("C1H"), a
10 chimeric 12G6 antibody, and humanized 12G6 antibodies to primary human T cells and huCD52 transgenic mouse T cells.

[0186] FIG. 33 is a table showing the relative binding efficiency of Campath-1H®, chimeric 2C3 and 12G6 antibodies, and humanized 2C3 and 12G6 antibodies to huCD52 expressing human and transgenic mouse T cells.

15 [0187] FIG. 34 illustrates the comparative binding patterns of humanized anti-CD52 Campath-1H®, 2C3, 12G6, and 9D9 antibodies to defined subsets of human peripheral blood mononuclear cell populations by flow cytometry. These histograms show that the humanized anti-CD52 antibody binding is equivalent to that of Campath-1H® for various CD52 expressing human PBMC subsets. The X axis represents the fluorescence emitted by the bound anti-CD52
20 antibody, while the area of each peak represents the total cell population.

[0188] FIG. 35 is a graph showing that chimeric and humanized 7F11 antibodies mediate equivalent ADCC activity on cells expressing CD52.

[0189] FIG. 36 is a graph showing that chimeric and humanized 7F11 antibodies mediate CDC activity on cells expressing CD52.

25 [0190] FIG. 37 is a graph showing that chimeric and humanized 2C3 antibodies mediate ADCC activity on cells expressing CD52.

[0191] FIG. 38 is a graph showing that chimeric and humanized 2C3 antibodies mediate CDC activity on cells expressing CD52.

30 [0192] FIG. 39 is a graph showing that chimeric and humanized 12G6 antibodies mediate ADCC activity on cells expressing CD52.

[0193] FIG. 40 is a graph showing that chimeric and humanized 12G6 antibodies mediate CDC activity on cells expressing CD52.

[0194] FIG. 41 is a graph showing that chimeric and humanized 9D9 antibodies mediate ADCC activity on cells expressing CD52.

[0195] FIG. 42 is a graph showing that chimeric and humanized 9D9 antibodies mediate CDC activity on cells expressing CD52.

5 [0196] FIG. 43 is a graph showing the ADCC activity of humanized anti-CD52 antibodies on primary T cells.

[0197] FIG. 44 is a graph showing the CDC activity of humanized anti-CD52 antibodies on primary T cells.

10 [0198] FIG. 45 is a graph showing neutralization of Campath-1H but not other anti-CD52 antibodies with CAMMS223 study human serum samples that contain anti-Campath-1H® neutralizing antibodies. Serum samples were taken from a representative patient (MS-1) at month 12 (M12) and month 13 (M13).

[0199] FIGS. 46A-46E show the level of CD4+ T cells, CD8+ T cells, B220+ B cells, NK cells, myeloid cells, and neutrophils in the blood 72 hours after dosing with Campath-1H® ("Campath") and humanized 4B10-H1/K1 ("4B10") antibodies.

[0200] FIGS. 47A-47E show the level of CD4+ T cells, CD8+ T cells, B220+ B cells, NK cells, myeloid cells, neutrophils, and macrophages in the spleen 72 hours after dosing with Campath-1H® ("Campath") and humanized 4B10-H1/K1 ("4B10") antibodies.

20 [0201] FIGS. 48A-48E show the levels of circulating cytokines 2 hours after dosing with Campath-1H® ("Campath") and humanized 4B10-H1/K1 ("4B10") antibodies.

[0202] FIGS. 49A and 49B show the repopulation of circulating lymphocytes over a time course after dosing with Campath-1H® ("Campath") and humanized 4B10-H1/K1 ("4B10") antibodies, (mg/kg).

25 [0203] FIGS. 50A-50E show the level of CD4+ T cells, CD8+ T cells, B220+ B cells, NK cells, myeloid cells, and neutrophils in the blood 72 hours after dosing with the humanized 7F11-SFD1/K2 ("7F11 SFD1") and 7F11-SFD2/K2 ("7F11 SFD2") antibodies.

[0204] FIGS. 51A-51E show the level of CD4+ T cells, CD8+ T cells, B220+ B cells, NK cells, myeloid cells, and neutrophils in the spleen 72 hours after dosing with the humanized 7F11-SFD1/K2 ("7F11 SFD1") and 7F11-SFD2/K2 ("7F11 SFD2") antibodies.

30 [0205] FIGS. 52A-52F show the levels of circulating cytokines 2 hours after dosing with the humanized 7F11-SFD1/K2 ("7F11 SFD1") and 7F11-SFD2/K2 ("7F11 SFD2") antibodies.

[0206] FIGS. 53A and 53B show the repopulation of circulating lymphocytes over a timecourse after dosing with the humanized 7F11-SFD1/K2 ("7F11 SFD1") and 7F11-SFD2/K2 ("7F11 SFD2") antibodies, (mg/kg).

5 [0207] FIGS. 54A and 54B show the level of CD4+ T cells, CD8+ T cells and B220+ B cells in the blood 72 hours after dosing with Campath-1H® ("Campath"), 7F11-chimeric antibodies, and humanized 7F11-SFD1/K2 and 7F11-SFD2/K2 antibodies.

[0208] FIG. 55 shows the level of Campath-1H® ("Campath"), 7F11-chimeric antibody and humanized 7F11-SFD1/K2 and 7F11-SFD2/K2 antibodies in the blood over a timecourse after dosing.

10 [0209] FIGS. 56A-56E show the level of CD4+ T cells, CD8+ T cells, B220+ B cells, NK cells, and neutrophils in the blood 72 hours after dosing with 2C3-SFD1/K12 antibodies.

[0210] FIGS. 57A-57E show the level of CD4+ T cells, CD8+ T cells, B220+ B cells, NK cells, and neutrophils in the spleen 72 hours after dosing with 2C3-SFD1/K12 antibodies.

15 [0211] FIGS. 58A-58F show the levels of circulating cytokines 2 hours after dosing with 2C3-SFD1/K12 ("2C3") antibodies.

[0212] FIG. 59 shows the repopulation of circulating lymphocytes over a timecourse after dosing with 2C3-SFD1/K12 antibodies, (mg/kg).

20 [0213] FIGS. 60A-60E show the level of CD4+ T cells, CD8+ T cells, B220+ B cells, NK cells, myeloid cells, macrophages, and neutrophils in the blood 72 hours after dosing with 12G6-SFD1/K11 antibodies.

[0214] FIGS. 61A-61E show the level of CD4+ T cells, CD8+ T cells, B220+ B cells, NK cells, macrophages, neutrophils, and myeloid cells in the spleen 72 hours after dosing with 12G6-SFD1/K11 antibodies.

25 [0215] FIGS. 62A-62F show the levels of circulating cytokines 2 hours after dosing with 12G6-SFD1/K11 ("12G6 hu") antibodies.

[0216] FIG. 63 shows the repopulation of circulating lymphocytes over a timecourse after dosing with 12G6-SFD1/K11 antibodies, (mg/kg).

30 [0217] FIGS. 64A-64C show the level of 2C3-chimeric, 2C3-SFD1/K12, 12G6-chimeric, 12G6-SFD1/K11, 9D9-chimeric, and 9D9-H10/K12 antibodies in the blood over a timecourse after dosing.

- [0218] FIGS. 65A-65E show the level of CD4+ T cells, CD8+ T cells, B220+ B cells, NK cells, myeloid cells, macrophages, and neutrophils in the blood 72 hours after dosing with 9D9-H10/K12 ("9D9") antibodies.
- [0219] FIGS. 66A-66E show the level of CD4+ T cells, CD8+ T cells, B220+ B cells, NK cells, myeloid cells, neutrophils, and macrophages in the spleen 72 hours after dosing with 9D9-H10/K12 ("9D9") antibodies.
- [0220] FIGS. 67A-67F show the levels of circulating cytokines 2 hours after dosing with 9D9-H10/K12 ("9D9") antibodies.
- [0221] FIG. 68 shows the repopulation of circulating lymphocytes over a timecourse after dosing with 9D9-H10/K12 ("9D9") antibodies, (mg/kg).
- [0222] FIGS. 69A-69D show the level of bulk lymphocyte populations (CD4+ T cells, CD8+ T cells, and B cells) and CD4+ T cell, CD8+ T cell, B220+ B cell and NK cell subtypes in the blood 72 hours after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K12 ("2C3"), 12G6-SFD1/K11 ("12G6"), and 9D9-H10/K12 ("9D9") antibodies.
- [0223] FIGS. 70A-70D show the level of bulk lymphocyte populations (CD4+ T cells, CD8+ T cells, and B cells) and CD4+ T cell, CD8+ T cell, B220+ B cell and NK cell subtypes in the spleen 72 hours after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K12 ("2C3"), 12G6-SFD1/K11 ("12G6"), and 9D9-H10/K12 ("9D9") antibodies.
- [0224] FIGS. 71A-71F show the levels of circulating cytokines 2 hours after dosing with Campath-1H®, 2C3-SFD1/K12, 12G6-SFD1/K11, and 9D9-H10/K12 antibodies.
- [0225] FIG. 72 shows the level of CD4+ T cells, CD8+ T cells, B220+ B cells, and NK cells in the blood 72 hours after dosing with 9D9-H10/K12 and 9D9-H11/K12 antibodies.
- [0226] FIG. 73 shows the level of CD4+ T cells, CD8+ T cells, B220+ B cells, and NK cells in the spleen 72 hours after dosing with 9D9-H10/K12 and 9D9-H11/K12 antibodies.
- [0227] FIGS. 74A-74D show the level of CD4+ T cells, CD8+ T cells, B220+ B cells, NK cells, and myeloid cells in the blood 72 hours after dosing with 12G6-SFD1/K11 ("12G6 K11") and 12G6-SFD1/K12 ("12G6 K12") antibodies.
- [0228] FIGS. 75A-75D show the level of CD4+ T cells, CD8+ T cells, B220+B cells, NK cells and myeloid cells in the spleen 72 hours after dosing with 12G6-SFD1/K11 ("12G6 K11") and 12G6-SFD1/K12 ("12G6 K12") antibodies.

[0229] FIG. 76 shows the level of bulk lymphocyte populations (CD4+ T cells, CD8+ T cells, and B220+ B cells) in the blood 72 hours after dosing with 9D9-H11/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies.

[0230] FIGS. 77A-77D show the level of CD4+ T cell, CD8+ T cell, B220+ B cell, NK cell, and myeloid cell subtypes in the blood 72 hours after dosing with 9D9-H11/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies.

[0231] FIG. 78 shows the level of bulk lymphocyte populations (CD4+ T cells, CD8+ T cells, and B220+ B cells) in the spleen 72 hours after dosing with 9D9-H11/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies.

[0232] FIGS. 79A-79D show the level of CD4+ T cell, CD8+ T cell, B220+ B cell, NK cell, and myeloid cell subtypes in the spleen 72 hours after dosing with 9D9-H11/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies.

[0233] FIGS. 80A-80F show the levels of circulating cytokines 2 hours after dosing with 9D9-H11/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies.

[0234] FIGS. 81A and 81B show the level of 2C3-SFD1/K12, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13 and 9D9-H18/K13 antibodies in the blood over a timecourse after dosing.

[0235] FIGS. 82A-82F show the level of cytokines in the blood over a 48-hour timecourse following dosing with Campath-1H® ("Campath"), 2C3-SFD1/K11, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13 or 9D9-H18/K13 antibodies.

[0236] FIGS. 83A-83E show the level of bulk lymphocytes, CD4+ T cells, CD8+ T cells, B220+ B cells, NK cells, and myeloid cells in the spleen 72 hours after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K11, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13 or 9D9-H18/K13 antibodies.

[0237] FIGS. 84A-84G show the repopulation of circulating CD4+ and CD8+ T cells, regulatory T cells, B cells, NK cells, neutrophils and macrophages over a timecourse after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K12, 9D9-H16/K13 and 12G6-SFD1/K12 antibodies.

[0238] FIG. 85 shows the ability of FITC-labeled Campath-1H® ("Campath"), 2C3-SFD1/K12 ("2C3 K12"), 12G6-SFD1/K11 ("12G6 K11"), 12G6-SFD1/K12 ("12G6 K12"), 9D9-H16/K13 ("9D9 H16"), and 9D9-H18/K13 ("9D9 H18") antibodies to specifically bind huCD52 lymphocyte cell populations in the spleen.

- [0239] FIGS. 86A-86E show the level of bulk lymphocyte populations (CD4+ T cells, CD8+ T cells, and B220+ B cells) and CD4+ T cell, CD8+ T cell, B220+ B cell, NK cell, and myeloid cell subtypes in the blood 72 hours after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K12, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies.
- [0240] FIGS. 87A-87E show the level of bulk lymphocyte populations (CD4+ T cells, CD8+ T cells, and B220+ B cells) and CD4+ T cell, CD8+ T cell, B220+ B cell, NK cell, and myeloid cell subtypes in the spleen 72 hours after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K12, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies.
- [0241] FIGS. 88A-88F show the levels of circulating cytokines 2 hours after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K12, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies.
- [0242] FIGS. 89A-89D show the level of CD4+ T cell, CD8+ T cell, B220+ B cell, and NK/myeloid cell subtypes in the blood 72 hours after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K12, 9D9-H16/K13 and 12G6-SFD1/K12 antibodies.
- [0243] FIGS. 90A-90D show the level of CD4+ T cell, CD8+ T cell, B220+ B cell, and NK/myeloid cell subtypes in the spleen 72 hours after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K12, 9D9-H16/K13 and 12G6-SFD1/K12 antibodies.
- [0244] FIGS. 91A-91D show the level of CD4+ T cell, CD8+ T cell, B220+ B cell, and NK/myeloid cell subtypes in the lymph node 72 hours after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K12, 9D9-H16/K13 and 12G6-SFD1/K12 antibodies.
- [0245] FIG. 92A shows the huCD52 expression level on CD4+ T cell, CD8+ T cell, B220+ B cell, and NK/myeloid cell subtypes in huCD52-KI/KO and non-transgenic control mice. FIG. 92B shows the huCD52 expression level on CD4+ T cells, CD8+ T cells, and B cells in huCD52-KI/KO and huCD52 CD1 transgenic mice.
- [0246] FIG. 93 shows the binding to huCD52 of 12G6-SFD1/K12 and 2C3-SFD1/K12 antibodies from various production sources ("small scale" and "large scale") as compared to a Campath-1H® control.
- [0247] FIG. 94 shows the level of bulk lymphocyte populations (CD4+ T cells, CD8+ T cells, B220+ B cells and NK cells) in the blood 72 hours after dosing with 12G6-SFD1/K12 and 2C3-SFD1/K12 antibodies from various production sources ("small scale" and "large scale").

[0248] FIG. 95 shows the levels of 2C3-SFD1/K12, 9D9-H16/K13 and 12G6-SFD1/K12 antibodies in the blood over a timecourse after dosing.

[0249] FIG. 96 demonstrates the EAE clinical score of 2C3-SFD1/K12 and 12G6-SFD1/K12 over a timecourse of disease progression.

5 [0250] FIGS. 97A and 97B demonstrate the ability of Campath1H® ("Campath"), 2C3-SFD1/K12 ("2C3"), His435Ala 2C3-SFD1/K12 ("H435A 2C3") and His310Ala/His435Gln 2C3-SFD1/K12 ("H310A/H435Q 2C3") to bind to mouse and human FcRn molecules.

10 [0251] FIG. 98 shows the in vivo clearance of 2C3-SFD1/K12 ("2C3 unmodified"), 2C3-SFD1/K12-Modified 1 ("2C3-Fc mutant 1") and 2C3-SFD1/K12-Modified 2 ("2C3-Fc mutant 2") in nontransgenic mice.

[0252] FIG. 99 shows the in vivo clearance of 2C3-SFD1/K12 ("2C3"), 2C3-SFD1/K12-Modified 1 ("2C3-Fc mutant 1") and 2C3-SFD1/K12-Modified 2 ("2C3-Fc mutant 2") in huCD52 transgenic mice.

15 [0253] FIGS. 100A and 100B show the level of bulk lymphocyte populations (CD4+ T cells, CD8+ T cells, B220+ B cells, and NK cells) in the blood and spleen 72 hours after dosing with 2C3-SFD1/K12 ("2C3"), 2C3-SFD1/K12-Modified 1 ("2C3 Fc mutant-1"), and 2C3-SFD1/K12-Modified 2 ("2C3 Fc mutant-2") antibodies.

20 [0254] FIGS. 101A and 101B are representative sensorgrams of Biacore T100 assays to determine the epitope specificity of the humanized 12G6-SFD1/K12 antibody and mutant peptides generated by alanine scanning. FIG. 101A shows no binding between 12G6-SFD1/K12 and the MUT 8 peptide, while FIG. 101B shows binding between 12G6-SFD1/K12 and the MUT 9 peptide.

25 [0255] FIG. 102 shows the TCR V beta analysis for donor BMS486. CD4+ T cells educated with Campath-1H® peptide group 986-989 exhibited preferential expansion of a single V beta (Vβ3).

[0256] FIG. 103 shows the TCR V beta analysis for donor BMS928. CD4+ T cells educated with 12G6-SFD1/K12 peptide groups 1066-67-68 and 1083-84-85 exhibited preferential expansion of a single V beta (Vβ20).

30 [0257] FIGS. 104A-104J show the Campath-1H® immunogenicity assessment. Proliferative responses are shown in CPM for individual donors A-J. The X axis depicts the groups of peptides used to stimulate autologous CD4+ T cells three times. Each group of T cells was

assayed in triplicate with autologous DCs pulsed with the educating antigen/peptide group (specific response, left bar, white), irrelevant DR binding peptide (middle bar, striped), or media (right bar, black).

[0258] FIGS. 105A-105J show the 12G6-SFD1/K12 immunogenicity assessment.

- 5 Proliferative responses are shown in CPM for individual donors A-J. The X axis depicts the groups of peptides used to stimulate autologous CD4+ T cells three times. Each group of T cells was assayed in triplicate with autologous DCs pulsed with the educating peptide group (specific response, left bar, white), irrelevant DR binding peptide (middle bar, striped), or media (right bar, black). In groups assayed without the media control, the left bar (white) represents DCs
- 10 pulsed with the educating peptide, and the right bar (striped) represents DCs pulsed with the irrelevant peptide.

- [0259]** FIG. 106 shows the full-length humanized heavy chain amino acid sequence of 2C3-SFD1 (SEQ ID NO: 272) and the full-length humanized light chain amino acid sequence of 2C3-K12 (SEQ ID NO: 273). The signal sequences are boldfaced and italicized and the CDRs
- 15 are underlined.

[0260] FIG. 107 shows the full-length humanized heavy chain amino acid sequence of 7F11-SFD1 (SEQ ID NO: 274) and the full-length humanized light chain amino acid sequence of 7F11-K2 (SEQ ID NO: 275). The signal sequences are boldfaced and italicized and the CDRs are underlined.

- 20 **[0261]** FIG. 108 shows the full-length humanized heavy chain amino acid sequences of 9D9-H16 (SEQ ID NO: 276) and 9D9-H18 (SEQ ID NO: 277), and the full-length humanized light chain amino acid sequence of 9D9-K13 (SEQ ID NO: 278). The signal sequences are boldfaced and italicized and the CDRs are underlined.

- [0262]** FIG. 109 shows the full-length humanized heavy chain amino acid sequence of 12G6-SFD1 (SEQ ID NO: 279) and the full-length humanized light chain amino acid sequence of 12G6-K12 (SEQ ID NO: 280). The signal sequences are boldfaced and italicized and the CDRs
- 25 are underlined.

- [0263]** FIG. 110 shows the full-length humanized heavy chain amino acid sequence of 4B10-H1 (SEQ ID NO: 281) and the full-length humanized light chain amino acid sequence of 4B10-K1 (SEQ ID NO: 282). The signal sequences are boldfaced and italicized and the CDRs are
- 30 underlined.

[0264] FIG. 111 shows the full-length humanized heavy chain nucleic acid sequence of 2C3-SFD1 (SEQ ID NO: 283) and the full-length humanized light chain nucleic acid sequence of 2C3-K12 (SEQ ID NO: 284). The signal sequences are underlined, the variable domains are in boldface, and the constant regions are italicized.

5 [0265] FIG. 112 shows the full-length humanized heavy chain nucleic acid sequence of 7F11-SFD1 (SEQ ID NO: 285) and the full-length humanized light chain nucleic acid sequence of 7F11-K2 (SEQ ID NO: 286). The signal sequences are underlined, the variable domains are in boldface, and the constant regions are italicized.

[0266] FIG. 113 shows the full-length humanized heavy chain nucleic acid sequences of 9D9-H16 (SEQ ID NO: 287) and 9D9-H18 (SEQ ID NO: 288). The signal sequences are underlined, the variable domains are in boldface, and the constant regions are italicized.

[0267] FIG. 114 shows the full-length humanized light chain nucleic acid sequence of 9D9-K13 (SEQ ID NO: 289). The signal sequence is underlined, the variable domain is in boldface, and the constant region is italicized.

15 [0268] FIG. 115 shows the full-length humanized heavy chain nucleic acid sequence of 12G6-SFD1 (SEQ ID NO: 290) and the full-length humanized light chain nucleic acid sequence of 12G6-K12 (SEQ ID NO: 291). The signal sequences are underlined, the variable domains are in boldface, and the constant regions are italicized.

[0269] FIG. 116 shows the full-length humanized heavy chain nucleic acid sequence of 4B10-H1 (SEQ ID NO: 292) and the full-length humanized light chain nucleic acid sequence of 4B10-K1 (SEQ ID NO: 293). The signal sequences are underlined, the variable domains are in boldface, and the constant regions are italicized.

DETAILED DESCRIPTION OF THE INVENTION

[0270] CD52 is a glycosylated, GPI anchored cell surface abundant protein (approximately 5×10^5 antibody binding sites per cell) present on at least 95% of all human peripheral blood lymphocytes and monocytes/macrophages (Hale G, et al., "The CAMPATH-1 antigen (CD52)," *Tissue Antigens*, 35:178-327 (1990)), but is absent from hematopoietic stem cells. This invention is directed to immunoglobulins (anti-CD52) which have binding specificity (*e.g.*, epitopic specificity) for, or are selective for binding to, human CD52 or a portion thereof. These immunoglobulins bind specifically to a CD52, and do not bind specifically to non-CD52 molecules. Specific binding between an anti-CD52 immunoglobulin and CD52 can be

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determined, for example, by measuring EC_{50} of the immunoglobulin's binding to CD52+ cells by flow cytometry. Specific binding can be indicated by an EC_{50} range of, e.g., 0.5 – 10 μ g/ml. The immunoglobulins described herein can have binding specificity for all or a portion of a human CD52 wherein the human CD52 is an isolated and/or recombinant human CD52, or on the surface of a cell which expresses human CD52. In addition, the immunoglobulins can have binding specificity for one or more forms of human CD52 (e.g., glycosylated human CD52; de-glycosylated human CD52; non-glycosylated human CD52; and allelic variants). In one embodiment, the immunoglobulins have binding specificity for a naturally occurring, endogenous or wildtype human CD52. The amino acid sequence of a wildtype human CD52 is set out in Figure 4 (SEQ ID NO: 104).

[0271] The immunoglobulins described herein can be purified or isolated using known techniques. Immunoglobulins that are "purified" or "isolated" have been separated away from molecules (e.g., peptides) of their source of origin (e.g., the supernatant of cells; in a mixture such as in a mixture of immunoglobulins in a library), and include immunoglobulins obtained by methods described herein or other suitable methods. Isolated immunoglobulins include substantially pure (essentially pure) immunoglobulins, and immunoglobulins produced by chemical synthesis, recombinant techniques and a combination thereof.

[0272] More specifically, the invention relates to anti-human CD52 immunoglobulins, antigen-binding fragments (i.e., portions) of the immunoglobulins, the light chains of the immunoglobulins, the heavy chains of the immunoglobulins, and fragments of these light chains or heavy chains. The invention also relates to mature immunoglobulins or chains thereof, such as glycosylated immunoglobulins. The invention also relates to immature or precursor immunoglobulin (protein). The invention also relates to nucleic acid molecules (e.g., vectors) that encode both these immature or mature proteins, to vectors and host cells that comprise such nucleic acid, to methods of producing immature and mature proteins and to methods of using the immunoglobulins.

[0273] The immunoglobulins of this invention can be used to treat a subject in need thereof (e.g., a human patient) to deplete the subject's lymphocytes and other CD52+ cells (e.g., CD52+ cancerous cells) as needed. As used herein, "lymphocyte depletion" is a type of immunosuppression by reducing the population of circulating lymphocytes, e.g., T cells and/or B cells, resulting in lymphopenia. The immunoglobulins of this invention can also be used to inhibit angiogenesis as further described below. The immunoglobulins of this invention also can

be used to enrich hematopoietic stem cells, for example, in *ex vivo* applications (*see, e.g.*, Lim et al., *J. Hematology & Oncology* 1:19 (2008)).

[0274] Naturally occurring immunoglobulins have a common core structure in which two identical light chains (about 24 kD) and two identical heavy chains (about 55 or 70 kD) form a tetramer. The amino-terminal portion of each chain is known as the variable (V) region and can be distinguished from the more conserved constant (C) regions of the remainder of each chain. Within the variable region of the light chain (also called the V_L domain) is a C-terminal portion known as the J region. Within the variable region of the heavy chain (also called the V_H domain), there is a D region in addition to the J region. Most of the amino acid sequence variation in immunoglobulins is confined to three separate locations in the V regions known as hypervariable regions or complementarity determining regions (CDRs) which are directly involved in antigen binding. Proceeding from the amino-terminus, these regions are designated CDR1, CDR2 and CDR3, respectively. The CDRs are held in place by more conserved framework regions (FRs). Proceeding from the amino-terminus, these regions are designated FR1, FR2, FR3 and FR4, respectively. The locations of CDR and FR regions and a numbering system have been defined by Kabat *et al.* (Kabat, E.A., *et al.*, *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, U.S. Government Printing Office (1991), Chothia & Lesk, *Canonical Structures for the Hypervariable Regions of Immunoglobulins*, *J. Mol. Biol.*, 196, 901-917 (1987), and the IMGT® numbering system (The International ImMunoGeneTics Information System®, Lefranc, M.-P., *The Immunologist* 7, 132-136 (1999)). Visual inspection and sequence analysis can be carried out to identify the CDR boundaries. For this invention, the CDR sequences are defined by using both the Kabat system and the IMGT system; that is, when the CDRs defined by the two systems do not entirely overlap, we include all the residues from the sequences defined by both systems.

[0275] Human immunoglobulins can be divided into classes and subclasses, depending on the isotype of the heavy chain. The classes include IgG, IgM, IgA, IgD and IgE, in which the heavy chains are of the gamma (γ), mu (μ), alpha (α), delta (δ) or epsilon (ε) type, respectively. Subclasses include IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2, in which the heavy chains are of the γ1, γ2, γ3, γ4, α1 and α2 type, respectively. Human immunoglobulin molecules of a selected class or subclass may contain either a kappa (κ) or lambda (λ) light chain. See *e.g.*, *Cellular and Molecular Immunology*, Wonsiewicz, M.J., Ed., Chapter 45, pp. 41-50, W. B. Saunders Co.,

Philadelphia, PA 91991); Nisonoff, A., *Introduction to Molecular Immunology*, 2nd Ed., Chapter 4, pp. 45-65, Sinauer Associates, Inc., Sunderland, MA (1984).

[0276] As used herein, the terms “immunoglobulin” and “antibody,” which are used interchangeably, refer to whole antibodies and antigen-binding fragments (i.e., “antigen-binding portions” – the two terms are used interchangeably herein unless otherwise indicated). Antigen-binding fragments of antibodies can be in the format of, for example, single chain antibodies, Fv fragments, Fab fragments, Fab’ fragments, F(ab’)₂ fragments, Fd fragments, single chain Fv molecules (scFv), bispecific single chain Fv dimers (PCT/US92/09665), diabodies, domain-deleted antibodies and single domain antibodies (dAbs). See *e.g.*, *Nature Biotechnology* 22(9):1161-1165 (2004)). Also within the invention are antigen-binding molecules comprising a VH and/or a VL. In the case of a VH, the molecule may also comprise one or more of a CH1, hinge, CH2 and CH3 region. Such single chain antibodies are also intended to be encompassed within the term “antigen-binding portion” of an antibody.

[0277] Antibody portion or fragments can be produced by enzymatic cleavage or by recombinant techniques. For instance, papain or pepsin cleavage can be used to generate Fab or F(ab’)₂ fragments, respectively. Antibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons have been introduced upstream of the natural stop site. For example, a recombinant construct encoding the heavy chain of an F(ab’)₂ fragment can be designed to include DNA sequences encoding the CH₁ domain and hinge region of the heavy chain. Preferred antigen-binding fragments have binding specificity for a wildtype human CD52.

[0278] In another aspect, the invention provides a variant of an antibody or portion thereof as described herein, wherein said variant binds to human CD52 specifically but differs from the reference antibody or portion thereof by 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions (for example, in a CDR region, a FR region, or a constant domain). For example, the variant antibody is at least 90%, at least 91%, at least 93%, at least 95%, at least 97% or at least 99% identical to the reference antibody in the heavy chain, the heavy chain variable domain, the light chain, or the light chain variable domain.

[0279] Sequence similarity or identity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For

instance, GCG contains programs such as “Gap” and “Bestfit” which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, e.g., GCG Version 6.1. Polypeptide sequences
5 also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, *Methods Enzymol.* 183:63-98 (1990); Pearson, *Methods Mol. Biol.* 132:185-219 (2000)). Another preferred algorithm when comparing a sequence of the invention to a database
10 containing a large number of sequences from different organisms is the computer program BLAST, especially blastp or tblastn, using default parameters. See, e.g., Altschul et al., *J. Mol. Biol.* 215:403-410 (1990); Altschul et al., *Nucleic Acids Res.* 25:3389-402 (1997); herein incorporated by reference.

[0280] According to the invention, one type of amino acid substitution that may be made is to
15 change one or more cysteines in the antibody, which may be chemically reactive, to another residue, such as, without limitation, alanine or serine. In one embodiment, there is a substitution of a non-canonical cysteine. The substitution can be made in a CDR or framework region of a variable domain or in the constant domain of an antibody. In some embodiments, the cysteine is canonical. Another type of amino acid substitution that may be made is to remove potential
20 proteolytic sites in the antibody. Such sites may occur in a CDR or framework region of a variable domain or in the constant domain of an antibody. Substitution of cysteine residues and removal of proteolytic sites may decrease the risk of heterogeneity in the antibody product and thus increase its homogeneity. Another type of amino acid substitution is to eliminate asparagine-glycine pairs, which form potential deamidation sites, by altering one or both of the
25 residues. In another aspect of the invention, the antibody may be deimmunized to reduce its immunogenicity using the techniques described in, e.g., PCT Publication WO98/52976 and WO00/34317.

[0281] Another type of amino acid substitution that may be made in one of the variants according to the invention is a conservative amino acid substitution. A “conservative amino
30 acid substitution” is one in which an amino acid residue is substituted by another amino acid residue having a side chain R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change

the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution.

Means for making this adjustment are well-known to those of skill in the art. See e.g., Pearson,

5 Methods Mol. Biol. 243:307-31 (1994).

[0282] Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine, and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side
10 chains: lysine, arginine, and histidine; 6) acidic side chains: aspartic acid and glutamic acid; and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix
15 disclosed in Gonnet et al., Science 256:1443-45 (1992). A “moderately conservative” replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0283] In certain embodiments, amino acid substitutions to an antibody or antigen-binding portion of the invention are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, for example,
20 to enhance ADCC and CDC activity of the antibody, (4) confer or modify other physicochemical or functional properties of such analogs, but still retain specific binding to human CD52, (5) remove C-terminal lysine, and (6) add or remove glycosylation sites.

[0284] In an aspect, the invention provides a new and novel polypeptide that is the heavy or light chain of an antibody of this invention, or that is a variable domain-containing portion of the
25 heavy or light chain. Such a polypeptide is useful because it can partner with an opposite (light or heavy) antibody chain to form a CD52-binding molecule.

Humanized Immunoglobulins

[0285] Described herein are humanized immunoglobulins comprising the CDRs of novel mouse anti-human CD52 antibodies. In one embodiment, the humanized immunoglobulin
30 comprises a humanized light chain and a humanized heavy chain that have CDR amino acid sequences which differ from the amino acid sequence of other humanized versions of anti-CD52 antibodies (e.g., Campath®).

[0286] The term "humanized immunoglobulin" as used herein refers to an immunoglobulin comprising chains that comprise one or more light chain CDRs (CDR1, CDR2 and CDR3) and one or more heavy chain CDRs (CDR1, CDR2 and CDR3) of an anti-CD52 antibody of non-human origin, also referred to herein as the donor antibody (*e.g.*, a murine anti-CD52 antibody),
5 and at least a portion of an immunoglobulin of human origin (*e.g.*, framework regions, or framework and constant regions, derived from a light and/or heavy chain of human origin, such as CDR-grafted antibodies with or without framework changes). The humanized immunoglobulin of the invention comprises at least one CDR that differs from at least one CDR (*e.g.*, from the corresponding CDR) present in Campath®. See, *e.g.*, Cabilly *et al.*, U.S. Patent
10 No. 4,816,567; Cabilly *et al.*, European Patent No. 0,125,023 B1; Boss *et al.*, U.S. Patent No. 4,816,397; Boss *et al.*, European Patent No. 0,120,694 B1; Neuberger, M.S. *et al.*, WO 86/01533; Neuberger, M.S. *et al.*, European Patent No. 0,194,276 B1; Winter, U.S. Patent No. 5,225,539; Winter, European Patent No. 0,239,400 B1; Padlan, E.A. *et al.*, European Patent Application No. 0,519,596 A1. See also, Ladner *et al.*, U.S. Patent No. 4,946,778; Huston, U.S.
15 Patent No. 5,476,786; and Bird, R.E. *et al.*, *Science*, 242: 423-426 (1988)), regarding single chain antibodies. In some embodiments, humanized immunoglobulins are de-immunized antibodies. See, *e.g.*, Carr *et al.*, U.S. Patent No. 7,264,806, regarding de-immunized immunoglobulins that have been modified to reduce the number of potential T-cell epitopes, thereby reducing the propensity for the immunoglobulin to elicit an immune response upon
20 administration to a human.

[0287] In particular embodiments, the humanized immunoglobulin comprises one or more light chain CDRs and one or more heavy chain CDRs of one or more of the following murine monoclonal antibodies: mouse 8G3.25.3.5, mouse 4G7.F3, mouse 9D9.A2, mouse 11C11.C5, mouse 3G7.E9, mouse 5F7.1.1.4, mouse 12G6.15.1.2, mouse 23E6.2.2.1, mouse 2C3.3.8.1,
25 mouse 7F11.1.9.7, and mouse 4B10.1.2.4.

[0288] In another embodiment, the humanized immunoglobulins bind human CD52 with an affinity similar to or better than that of Campath®. In a particular embodiment, the humanized immunoglobulin of the present invention has the binding specificity of a murine anti-human CD52 antibody of the invention (*e.g.*, having specificity for human CD52, having the same or
30 similar epitopic specificity) and/or it has the same inhibitory function. The humanized immunoglobulins can have the binding specificity and/or inhibitory activity of a murine anti-human CD52 antibody or humanized anti-human CD52 antibody described herein, and/or the

epitopic specificity of a murine anti-human CD52 antibody or humanized anti-human CD52 antibody described herein (*e.g.*, it can compete with the murine anti-human CD52 antibody, or another humanized anti-CD52 antibody (*e.g.*, Campath®) for binding to CD52, and/or it can have the inhibitory function of the murine or humanized anti-human CD52 antibody). In a particular embodiment, the humanized immunoglobulin has the binding specificity, epitopic specificity and/or inhibitory activity of any one of mouse antibodies 8G3, 4G7, 9D9, 11C11, 3G7, 5F7, 12G6, 23E6, 2C3, 7F11, and 4B10.

[0289] The portion of the humanized immunoglobulin or immunoglobulin chain which is of human origin (*e.g.*, framework region; constant region) can be derived from any suitable human immunoglobulin or immunoglobulin chain. For example, a human constant region or portion thereof in a humanized or chimeric antibody can be derived from a human κ or λ light chain gene, and/or from a human γ (*e.g.*, $\gamma 1$, $\gamma 2$, $\gamma 3$, $\gamma 4$), μ , α (*e.g.*, $\alpha 1$, $\alpha 2$), δ or ϵ heavy chain gene, including allelic variants. A particular constant region (*e.g.*, IgG1), variant or portion thereof can be selected in order to tailor effector function. For example, a mutated constant region (variant) can be incorporated into the immunoglobulin or immunoglobulin chain so as to minimize binding to Fc receptors and/or ability to fix complement. (See *e.g.*, Winter *et al.*, GB 2,209,757 B; Morrison *et al.*, WO 89/07142; Morgan *et al.*, WO 94/29351, December 22, 1994). In one embodiment, the human framework has no variation or mutation in its structure or sequence. In a particular embodiment, the framework is a germline framework sequence that has no mutations or variations in its sequence.

[0290] As used herein, the term "germline" refers to the nucleotide sequences and amino acid sequences of the antibody genes and gene segments as they are passed from parents to offspring via the germ cells. This germline sequence is distinguished from the nucleotide sequences encoding antibodies in mature B cells which have been altered by recombination and hypermutation events during the course of B cell maturation. An antibody that "utilizes" a particular germline has a nucleotide or amino acid sequence that most closely aligns with that germline nucleotide sequence or with the amino acid sequence that it specifies. Such antibodies frequently are mutated compared with the germline sequence.

[0291] In other embodiments, the human framework has minimal variation or mutation from germline sequence in its structure or sequence (*e.g.*, less than 3, 4, 5, 6, 7, 8, 9, or 10 acceptor framework residues have been replaced with donor framework residues to improve binding affinity, see Queen *et al.*, U.S. Patent No. 5,530,101). In a particular embodiment, a limited

number of amino acids in the framework of a humanized immunoglobulin chain (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids) are chosen to be the same as the amino acids at those positions in the donor sequence (*i.e.*, “back-mutated”), rather than in the acceptor sequence, to increase the affinity of an antibody comprising the humanized immunoglobulin chain for human CD52.

5 [0292] Human framework regions (*e.g.*, of the heavy and/or light chain variable regions) are preferably obtained or derived from a human antibody variable region having sequence similarity to the analogous or equivalent region (*e.g.*, heavy or light chain variable regions) of the antigen-binding region of the donor immunoglobulin (murine anti-CD52 antibody). Other sources of framework regions for portions of human origin of a humanized immunoglobulin
10 include human variable region consensus sequences (See *e.g.*, Kettleborough, C. A. *et al.*, *Protein Engineering* 4:773-783 (1991); Carter *et al.*, WO 94/04679; Carter U.S. Patent 6,407,213)). For example, the region of the donor sequence of the antibody (*e.g.*, the sequence of the variable region) used to obtain the nonhuman portion can be compared to human sequences as described in Kabat, E. A. *et al. Sequences of Proteins of Immunological Interest*,
15 Fifth Edition, U.S. Department of Health and Human Services, U.S. Government Printing Office (1991) to select a particular source of the human portions of the humanized immunoglobulin, *e.g.*, a source of the framework regions.

[0293] In one embodiment, the framework regions of the humanized immunoglobulin chains are obtained, or derived, from a human Ig variable region having at least about 50%, at least
20 about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% or at least about 95% overall sequence identity, with the variable region of the nonhuman donor. In a particular embodiment, the framework regions of the humanized immunoglobulin chains are obtained or derived from human variable region framework regions having at least about 50%, at least about 55%, at least
25 about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% overall sequence identity, with the framework regions of the variable region of the nonhuman donor immunoglobulin.

[0294] In one embodiment, at least one of the framework regions (FR) of the humanized immunoglobulin is obtained or derived from one or more chains of an antibody of human origin.
30 Thus, the FR can include a FR1 and/or FR2 and/or FR3 and/or FR4 obtained or derived from one or more antibodies of human origin (*e.g.*, from a human immunoglobulin chain, from a human consensus sequence).

- [0295] The immunoglobulin portions for use in the present invention have sequences identical, or similar, to immunoglobulins from which they are derived or to variants thereof. Such variants include mutants differing by the addition, deletion or substitution (*e.g.*, conservative substitution) of one or more residues, *e.g.*, differing by up to 3, 4, 5, 6, 7, 8, 9, or 10 residues from the parental sequence by one or more additions, deletions or substitutions. As indicated above, the humanized immunoglobulin of the invention comprises one or more CDRs from one or more of the murine anti-CD52 antibodies (donor antibodies) described herein. Changes in the framework region, such as those which substitute a residue of the framework region of human origin with a residue from the corresponding position of the donor antibody, can be made. One or more mutations, including deletions, insertions and substitutions of one or more amino acids in the framework region, can be made. If desired, framework mutations can be included in a humanized antibody or chain, and sites for mutation can be selected using any suitable method, for example as described in WO 98/06248, the entire teachings of which are incorporated by reference.
- [0296] It will be appreciated by one of skill in the art that in some cases residues flanking the one or more CDRs of the murine anti-CD52 antibody(ies) may contribute, and in some cases, may be essential, either directly or indirectly, to function (*e.g.*, binding). Thus, in some embodiments, one or more amino acids flanking one or more CDRs (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 flanking amino acids) of the murine framework are also included in the humanized immunoglobulin.
- [0297] In some embodiments, the human heavy chain framework regions of the humanized antibodies of this invention utilize the human VH3-72 or VH3-23 germline sequence. In some embodiments, the human light chain framework regions of the humanized antibodies of this invention utilize the human Vk2-A18b germline sequence. Back mutations may optionally be made in these FR regions at one or more of the residues as described in the Working Examples below to improve CD52-binding affinity of the humanized antibody.
- [0298] "Affinity" is a term of art that describes the strength of a binding interaction and typically refers to the overall strength of binding of the immunoglobulin to human CD52.
- [0299] In a particular embodiment, the immunoglobulin has a binding activity measured as an EC₅₀ value of less than 10 µg/ml (*e.g.*, as determined by flow cytometry). In another embodiment, the immunoglobulin has a binding activity measured as an EC₅₀ value of less than 5.0 µg/ml, or less than 1.0 µg/ml (*e.g.*, as determined by flow cytometry).

[0300] In some embodiments, the immunoglobulin binds to human CD52 with an affinity (K_D ; $K_D = K_{off} (k_d) / K_{on} (k_a)$) of 300 nM to 1 pM (i.e., 3×10^{-7} to 1×10^{-12} M), preferably 50 nM to 1 pM, more preferably 5 nM to 1 pM and most preferably 1 nM to 1 pM, for example, a K_D of 1×10^{-7} M or less, preferably 1×10^{-8} M or less, more preferably 1×10^{-9} M or less, advantageously 1×10^{-10} M or less and most preferably 1×10^{-11} M or 1×10^{-12} or less; and/or a K_{off} rate constant of 5×10^{-1} s⁻¹ to 1×10^{-7} s⁻¹, preferably 1×10^{-2} s⁻¹ to 1×10^{-6} s⁻¹, more preferably 5×10^{-3} s⁻¹ to 1×10^{-5} s⁻¹, for example 5×10^{-1} s⁻¹ or less, preferably 1×10^{-2} s⁻¹ or less, advantageously 1×10^{-3} s⁻¹ or less, more preferably 1×10^{-4} s⁻¹ or less, still more preferably 1×10^{-5} s⁻¹ or less, and most preferably 1×10^{-6} s⁻¹ or less as determined by surface plasmon resonance.

[0301] As is apparent to one of skill in the art, a variety of methods can be used to confirm that immunoglobulins produced according to methods provided herein and known in the art have the requisite specificity (e.g., binding specificity, epitopic specificity). For example, the binding function of a humanized anti-CD52 immunoglobulin of the invention having binding specificity for human CD52 can be detected using any suitable method, e.g., assays which monitor formation of a complex between humanized immunoglobulin and human CD52 (e.g., a membrane fraction comprising human CD52; a cell bearing human CD52, such as a human T cell, a human B cell; a CHO cell or a recombinant host cell comprising and expressing a nucleic acid encoding human CD52; a peptide (e.g., a synthetic peptide) having an amino acid sequence of CD52; a solid support comprising human CD52).

[0302] The ability of an immunoglobulin of the invention (e.g., a humanized immunoglobulin of the invention) to bind to the same epitope on human CD52 as a particular murine, chimeric, or humanized monoclonal antibody, or to bind to an epitope on human CD52 which overlaps with the epitope on human CD52 to which a particular murine, chimeric, or humanized monoclonal antibody binds, can be readily determined using a variety of techniques known to those of skill in the art, including e.g., competitive binding assays. These may involve the use of a labeled form of said particular antibody, and a measurement of the binding of that labeled antibody to human CD52 in the presence and in the absence of an immunoglobulin of the invention.

[0303] An "epitope" as used herein includes any protein determinant capable of specific binding to an immunoglobulin. Epitopic determinants generally consist of chemically active surface groupings of molecules such as amino acids or carbohydrate or sugar side chains and

generally have specific three dimensional structural characteristics, as well as specific charge characteristics. An epitope may be "linear" or "conformational." In a linear epitope, all of the points of interaction between the protein and the interacting molecule (such as an antibody) occur linearly along the primary amino acid sequence of the protein. In a conformational epitope, the points of interaction occur across amino acid residues on the protein that are separated from one another. Once a desired epitope on an antigen is determined, it is possible to generate antibodies to that epitope, e.g., using the techniques described in the present invention. Alternatively, during the discovery process, the generation and characterization of antibodies may elucidate information about desirable epitopes. From this information, it is then possible to competitively screen antibodies for binding to the same epitope. An approach to achieve this is to conduct competition studies to find antibodies that competitively bind with one another, i.e., the antibodies compete for binding to the antigen.

[0304] In one embodiment, to determine if a test antibody binds to the same or overlapping epitope of a humanized antibody of this invention, one allows the anti-CD52 antibody of the invention to bind to CD52 under saturating conditions and then measures the ability of the test antibody to bind to CD52. If the test antibody is able to bind to CD52 at the same time as the reference anti-CD52 antibody, then the test antibody binds to a different epitope than the reference anti-CD52 antibody. However, if the test antibody is not able to bind to CD52 at the same time, then the test antibody binds to the same epitope, an overlapping epitope, or an epitope that is in close proximity to the epitope bound by the anti-CD52 antibody of the invention. This experiment can be performed using ELISA, RIA, BIACORE™, or flow cytometry. To test whether an anti-CD52 antibody cross-competes with another anti-CD52 antibody, one may use the competition method described above in two directions, i.e., determining if the reference antibody blocks the test antibody and vice versa. In a some embodiment, the experiment is performed using BIACORE™.

[0305] Epitope binning can also be useful to characterize the antibodies of this invention. The term "binning" refers to a method to group antibodies based on their antigen binding characteristics. A high throughput process for "binning" antibodies based upon their cross-competition is described in International Patent Application No. WO 03/48731. The "epitope binning" can be investigated by allowing an unlabeled form of an anti-CD52 antibody "A" to bind to a synthetic peptide corresponding to the sequence of CD52 or to CD52 positive cells. Subsequently a labeled second anti-CD52 antibody "B" is added and one can assess the amount

of labeled antibody that can bind relative to a control sample where the cells or synthetic peptide have not been exposed previously to anti-CD52 antibody "A." Alternatively, anti-CD52 antibodies "A" and "B" can both be labeled with different fluorochromes or chemicals enabling detection, and one can measure the quantities of both labeled antibodies that can engage the

5 CD52 peptide at the same time using a device capable of detecting the label or measure the amounts of both antibodies that simultaneously engage CD52 positive cells by flow cytometry. Biacore and Octet technologies enable one to investigate the competitive binding of unlabelled forms of antibodies. This use of unlabelled forms of antibodies is desired as the chemical modification of some antibodies can compromise the binding activity. See also the technology

10 described in *See also Jia et al., J. Immunol. Methods* 288:91-98 (2004), which is useful in performing epitope binning as well.

[0306] Also provided herein are portions of the humanized immunoglobulins such as light chains, heavy chains and portions of light and heavy chains. These immunoglobulin portions can be obtained or derived from immunoglobulins (*e.g.*, by reduction and/or cleavage), or

15 produced or expressed by nucleic acids encoding a portion of an immunoglobulin or chain thereof having the desired property (*e.g.*, binds human CD52, sequence similarity). They can be prepared by *e.g.*, *de novo* synthesis of the relevant portion. Humanized immunoglobulins comprising the desired portions (*e.g.*, antigen-binding region, CDR, FR, C region) of human and nonhuman origin can be produced using synthetic and/or recombinant nucleic acids to prepare

20 constructs (*e.g.*, cDNA) encoding the desired humanized chain. For example, to prepare a portion of an immunoglobulin (*e.g.*, a portion of a chain), one or more stop codons can be introduced at the desired position. Nucleic acid (*e.g.*, DNA) sequences coding for humanized variable regions can be constructed using PCR mutagenesis methods to alter existing DNA sequences (see *e.g.*, Kamman, M., *et al.*, *Nucl. Acids Res.* 17:5404 (1989)). PCR primers coding

25 for the new CDRs can be hybridized to a DNA template of a previously humanized variable region which is based on the same, or a very similar, human variable region (Sato, K., *et al.*, *Cancer Research* 53:851-856 (1993)). If a similar DNA sequence is not available for use as a template, a nucleic acid comprising a sequence encoding a variable region sequence can be constructed from synthetic oligonucleotides (see *e.g.*, Kolbinger, F., *Protein Engineering* 8:971-

30 980 (1993)). A sequence encoding a signal peptide can also be incorporated into the nucleic acid (*e.g.*, on synthesis, upon insertion into a vector). If a signal peptide sequence is unavailable (*e.g.*, not typically present), a signal peptide sequence from another antibody can be used (see,

e.g., Kettleborough, C.A., *Protein Engineering* 4:773-783 (1991)). Using these methods, methods described herein or other suitable methods, variants can readily be produced.

[0307] The invention relates to a humanized immunoglobulin that has binding specificity for human CD52 and comprises a humanized light chain and a humanized heavy chain and/or portions thereof. In one embodiment, the humanized immunoglobulin comprises a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 3 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 16; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 4 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 17; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 5 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 18; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 6 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 19; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 7 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 20; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 8 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 21; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 9 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 22; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 10 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 23; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 11 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 24; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 12 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 25; or a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 13 and a heavy chain sequence comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 26.

[0308] In one embodiment, a humanized immunoglobulin of the invention comprises heavy chain (H)-CDR1, H-CDR2, H-CDR3, light chain (L)-CDR1, L-CDR2, and L-CDR3 whose amino acid sequences are: a) SEQ ID NOs: 51, 59, 69, 29, 36, and 43, respectively; b) SEQ ID NOs: 50, 60, 69, 29, 37, and 43, respectively; c) SEQ ID NOs: 50, 61, 68, 29, 38, and 43, respectively; d) SEQ ID NOs: 50, 61, 69, 29, 36, and 43, respectively; e) SEQ ID NOs: 50, 62,

69, 29, 39, and 43, respectively; f) SEQ ID NOs: 52, 61, 70, 30, 40, and 43, respectively; g) SEQ ID NOs: 53, 63, 71, 31, 36, and 44, respectively; h) SEQ ID NOs: 54, 64, 71, 31, 36, and 45, respectively; i) SEQ ID NOs: 55, 63, 72, 31, 36, and 46, respectively; j) SEQ ID NOs: 56, 65, 73, 32, 41, and 47, respectively; k) SEQ ID NOs: 56, 65, 294, 32, 41, and 47; or l) SEQ ID NOs: 56, 66, 74, 33, 41, and 48, respectively.

[0309] In another embodiment, a humanized immunoglobulin of this invention comprises H-CDR3 and L-CDR3 whose sequences are a) SEQ ID NOs: 69 and 43, respectively; b) SEQ ID NOs: 68 and 43, respectively; c) SEQ ID NOs: 70 and 43, respectively; d) SEQ ID NOs: 71 and 44, respectively; e) SEQ ID NOs: 71 and 45, respectively; f) SEQ ID NOs: 72 and 46, respectively; g) SEQ ID NOs: 73 and 47, respectively; h) SEQ ID NOs: 294 and 47, respectively; or i) SEQ ID NOs: 74 and 48, respectively.

[0310] In another embodiment, the humanized immunoglobulin has binding specificity for human CD52 and comprises a light chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, and SEQ ID NO: 48, or a combination thereof; and a heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, and SEQ ID NO: 294, or a combination thereof, wherein the humanized immunoglobulin is not Campath®.

[0311] In another embodiment, the humanized immunoglobulin that has a binding specificity for human CD52 comprises a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 or SEQ ID NO: 13, and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22,

SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, or SEQ ID NO: 137, wherein the humanized immunoglobulin is not Campath®.

[0312] The invention also relates to a humanized immunoglobulin light chain of the humanized immunoglobulin described herein. In one embodiment, the humanized immunoglobulin light chain comprises one or more CDRs selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, and SEQ ID NO: 48, and a combination thereof, wherein the humanized immunoglobulin light chain is not the light chain of Campath®. For example, the humanized antibody has L-CDR1, L-CDR2, and L-CDR3 whose amino acid sequences are: a) SEQ ID NOs: 29, 36, and 43, respectively; b) SEQ ID NOs: 29, 37, and 43, respectively; c) SEQ ID NOs: 29, 38, and 43, respectively; d) SEQ ID NOs: 29, 36, and 43, respectively; e) SEQ ID NOs: 29, 39, and 43, respectively; f) SEQ ID NOs: 30, 40, and 43, respectively; g) SEQ ID NOs: 31, 36, and 44, respectively; h) SEQ ID NOs: 31, 36, and 45, respectively; i) SEQ ID NOs: 31, 36, and 46, respectively; j) SEQ ID NOs: 32, 41, and 47, respectively; or k) SEQ ID NOs: 33, 41, and 48, respectively.

[0313] The invention also relates to humanized heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, and SEQ ID NO: 294, or a combination thereof, wherein the humanized immunoglobulin heavy chain is not the heavy chain of Campath®. For example, the humanized antibody has H-CDR1, H-CDR2, and H-CDR3 whose amino acid sequences are: a) SEQ ID NOs: 51, 59, and 69, respectively; b) SEQ ID NOs: 50, 60, and 69, respectively; c) SEQ ID NOs: 50, 61, and 68, respectively; d) SEQ ID NOs: 50, 61, and 69, respectively; e) SEQ ID NOs: 50, 62, and 69, respectively; f) SEQ ID NOs: 52, 61, and 70, respectively; g) SEQ ID NOs: 53, 63, and 71, respectively; h) SEQ ID NOs: 54, 64, and 71, respectively; i) SEQ ID NOs: 55, 63, and 72, respectively; j) SEQ ID NOs: 56, 65, and 73, respectively; k) SEQ ID NOs: 56, 65, and 294; or l) SEQ ID NOs: 56, 66, and 74, respectively.

[0314] In one embodiment, a humanized antibody of this invention comprises a light chain comprising a variable domain (V_L) sequence of one of SEQ ID NOs: 102, 138, 145-148, 153-157, and 164-168. In a related embodiment, the humanized antibody comprises a light chain whose amino acid sequence comprises or consists of one of SEQ ID NOs: 273, 275, 278, 280, and 282.

[0315] In one embodiment, a humanized antibody of this invention comprises a heavy chain comprising a variable domain (V_H) sequence of one of SEQ ID NOs: 103, 136, 137, 139-144, 149-152, and 158-163. In a related embodiment, the humanized antibody comprises a heavy chain whose amino acid sequence comprises or consists of one of SEQ ID NOs: 272, 274, 276, 277, 279, and 281.

[0316] In some embodiments, a humanized antibody of this invention comprises a V_H and a V_L whose amino acid sequences comprise or consist of

- a) SEQ ID NOs: 103 and 102, respectively (4B10-H1/K1);
- b) SEQ ID NOs: 136 and 138, respectively (7F11-SFD1/K2);
- c) SEQ ID NOs: 137 and 138, respectively (7F11-SFD2/K2)
- d) SEQ ID NO: 139 and one of SEQ ID NOs: 145-148, respectively (e.g., SEQ ID NOs: 139 and 146, respectively (2C3-SFD1/K11); and SEQ ID NOs: 139 and 147, respectively (2C3-SFD1/K12));
- e) SEQ ID NO: 140 and one of SEQ ID NOs: 145-148, respectively;
- f) SEQ ID NO: 141 and one of SEQ ID NOs: 145-148, respectively;
- g) SEQ ID NO: 142 and one of SEQ ID NOs: 145-148, respectively;
- h) SEQ ID NO: 143 and one of SEQ ID NOs: 145-148, respectively;
- i) SEQ ID NO: 144 and one of SEQ ID NOs: 145-148, respectively;
- j) SEQ ID NO: 149 and one of SEQ ID NOs: 153-157, respectively (e.g., SEQ ID NOs: 149 and 155, respectively (12G6-SFD1/K11); SEQ ID NOs: 149 and 156, respectively (12G6-SFD1/K12); and SEQ ID NOs: 149 and 157, respectively (12G6-SFD1/K13));
- k) SEQ ID NO: 150 and one of SEQ ID NOs: 153-157, respectively;
- l) SEQ ID NO: 151 and one of SEQ ID NOs: 153-157, respectively;
- m) SEQ ID NO: 152 and one of SEQ ID NOs: 153-157, respectively;
- n) SEQ ID NO: 158 and one of SEQ ID NOs: 164-168, respectively (e.g., SEQ ID NOs: 158 and 165, respectively (9D9-H10/K12); and SEQ ID NOs: 158 and 166, respectively (9D9-H10/K13));

o) SEQ ID NO: 159 and one of SEQ ID NOs: 164-168, respectively (e.g., SEQ ID NOs: 159 and 165, respectively (9D9-H11/K12); and SEQ ID NOs: 159 and 166, respectively (9D9-H11/K13));

p) SEQ ID NO: 160 and one of SEQ ID NOs: 164-168, respectively;

5 q) SEQ ID NO: 161 and one of SEQ ID NOs: 164-168, respectively (e.g., SEQ ID NOs: 161 and 166, respectively (9D9-H16/K13));

r) SEQ ID NO: 162 and one of SEQ ID NOs: 164-168, respectively; or

s) SEQ ID NO: 163 and one of SEQ ID NOs: 164-168, respectively (e.g., SEQ ID NOs: 163 and 166, respectively (9D9-H18/K13)).

10 The antibodies included in the parentheses are further described below in the working examples.

[0317] In one embodiment, a humanized antibody of this invention comprises a light chain (LC) and a heavy chain (HC) whose amino acid sequences comprise or consist of a) SEQ ID NOs: 273 and 272, respectively; b) SEQ ID NOs: 275 and 274, respectively; c) SEQ ID NOs: 278 and 276, respectively; d) SEQ ID NOs: 278 and 277, respectively; e) SEQ ID NOs: 280 and 279, respectively; or f) SEQ ID NOs: 282 and 281, respectively.

[0318] This invention also provides anti-human CD52 antibodies (except those, in any, known in the prior art) that binds to the same epitope as, or competes or cross-competes with, an antibody exemplified herein. These antibodies can be, for example, humanized, chimeric, or mouse antibodies. For example, the invention provides anti-human CD52 antibodies that bind to the same epitope as, or competes or cross-competes with, one of mouse antibodies 8G3, 4F7, 9D9, 11C11, 3G7, 5F7, 12G6, 23E6, 2C3, 7F11, and 4B10, and humanized and chimeric versions of these mouse antibodies. The ability of an antibody to bind to the same epitope as, or competes or cross-competes with a reference antibody can be determined as described above. For example, we have found that the CD52 epitope bound by the humanized antibodies 2C3-SFD1/K12 and 12G6-SFD1/K12 includes residues 7, 8, and 11 in SEQ ID NO: 104, and that the epitope bound by the humanized antibody 9D9-H16/K13 includes residues 4 and 11 in SEQ ID NO: 104. Thus, in some embodiments, this invention provides anti-CD52 antibodies that bind to the same epitope as, or competes or cross-competes with, those humanized antibodies.

[0319] If desired, for example, for diagnostic or assay purposes (e.g., imaging to allow, for example, monitoring of therapies), the humanized immunoglobulin (e.g., antigen-binding fragment thereof) can comprise a detectable label. Suitable detectable labels and methods for labeling a humanized immunoglobulin or antigen-binding fragment thereof are well known in

the art. Suitable detectable labels include, for example, a radioisotope (*e.g.*, as Indium-111, Technetium-99m or Iodine-131), positron emitting labels (*e.g.*, Fluorine-19), paramagnetic ions (*e.g.*, Gadolinium (III), Manganese (II)), an epitope label (tag), an affinity label (*e.g.*, biotin, avidin), a spin label, an enzyme, a fluorescent group or a chemiluminescent group. When labels are not employed, complex formation (*e.g.*, between humanized immunoglobulin and human CD52) can be determined by surface plasmon resonance, ELISA, FACS, or other suitable methods.

[0320] Anti-CD52 antibodies used in the invention also may be conjugated, via, for example, chemical reactions or genetic modifications, to other moieties (*e.g.*, pegylation moieties) that improve the antibodies' pharmacokinetics such as half-life. In some embodiments, the anti-CD52 antibodies used in this invention can be linked to a suitable cytokine via, *e.g.*, chemical conjugation or genetic modifications (*e.g.*, appending the coding sequence of the cytokine in frame to an antibody coding sequence, thereby creating an antibody:cytokine fusion protein).

[0321] The invention also relates to immunoconjugates in which the humanized immunoglobulin (*e.g.*, antigen-binding fragment thereof) of the invention is coupled to another therapeutic agent, such as a bioactive compound (*e.g.*, cytokines, superantigens, cytotoxic agents and toxins). For example, the humanized immunoglobulin that has binding specificity for human CD52 (*e.g.*, antigen binding fragment thereof) can be coupled to a biological protein, a molecule of plant or bacterial origin (or derivative thereof), an interleukin-2 antibody or diphtheria toxin antibodies.

Mouse Monoclonal Immunoglobulins

[0322] As described herein, mouse monoclonal immunoglobulins having binding specificity for human CD52 have been produced. Humanized and chimeric antibodies of this invention can be derived from the mouse monoclonal antibodies of this invention. That is, in some embodiments, humanized and chimeric anti-CD52 antibodies of the invention comprise sequences taken from a mouse monoclonal antibody of the invention, such as one or more CDR sequences. A mouse monoclonal immunoglobulin of this invention comprises a light chain and a heavy chain that have CDR amino acid sequences which differ from the CDR amino acid sequences of known mouse anti-CD52 monoclonal antibodies (*e.g.*, from CF1D12).

[0323] As used herein, the term "mouse monoclonal immunoglobulin" refers to an immunoglobulin containing light chain CDRs (L-CDR1, L-CDR2 and L-CDR3) and heavy chain CDRs (H-CDR1, H-CDR2 and H-CDR3) of a murine anti-human CD52 antibody, and

framework and constant regions of murine origin. Mouse monoclonal immunoglobulins are homogeneous antibodies of a single specificity prepared, for example, by the use of hybridoma technology or recombinant methods.

[0324] The invention relates to the mouse monoclonal immunoglobulins described herein, including antigen-binding fragments (i.e., portions) of the mouse monoclonal immunoglobulins, the light chains of the mouse monoclonal immunoglobulins, the heavy chains of the mouse monoclonal immunoglobulins, and fragments of these heavy and light chains. In a particular embodiment, the mouse monoclonal antibody is the mouse 8G3.25.3.5 (also called GENZ 8G3.25.3.5 or 8G3), mouse GMA 4G7.F3 (also called 4G7.F3 or 4G7), mouse GMA 9D9.A2 (also called 9D9.A2 or 9D9), mouse GMA 11C11.C5 (also called 11C11.C5 or 11C11), mouse GMA 3G7.E9 (also called 3G7.E9 or 3G7), mouse 5F7.1.1.4 (also called GENZ 5F7.1.1.4 or 5F7), mouse 12G6.15.1.2 (also called GENZ 12G6.15.1.2 or 2G6), mouse 23E6.2.2.1 (also called GENZ 23E6.2.2.1 or 23E6), mouse 2C3.3.8.1 (also called GENZ 2C3.3.8.1 or 2C3), mouse 7F11.1.9.7 (also called GENZ 7F11.1.9.7 or 7F11), or mouse 4B10.1.2.4 (also called GENZ 4B10.1.2.4 or 4B10). The invention relates to mature mouse monoclonal immunoglobulin, such as the mouse monoclonal immunoglobulin following processing to remove the heavy and light chain signal peptides and/or to the glycosylated immunoglobulin. The invention also relates to immature or precursor protein, such as a mouse immunoglobulin light chain or a mouse immunoglobulin heavy chain comprising a signal peptide. The invention also relates to nucleic acid molecules (e.g., vectors) that encode these immature or mature proteins, to host cells that comprise such nucleic acids and to methods of producing these immature and mature proteins.

[0325] The binding function of a mouse monoclonal immunoglobulin having binding specificity for human CD52 can be detected using any suitable method, for example using assays which monitor formation of a complex between mouse monoclonal immunoglobulin and human CD52 (e.g., a membrane fraction comprising human CD52, or a cell bearing human CD52, such as a human T cell, a human B cell, CHO cell or a recombinant host cell comprising a nucleic acid encoding human CD52; a peptide (e.g., a synthetic peptide) having an amino acid sequence of CD52).

[0326] Also provided herein are portions of the murine immunoglobulins which include light chains, heavy chains and portions of light and heavy chains. These immunoglobulin portions can be obtained or derived from immunoglobulins (e.g., by reduction and/or cleavage), or

nucleic acids encoding a portion of an immunoglobulin or chain thereof having the desired property (*e.g.*, binds human CD52, sequence similarity) can be produced and expressed. They can be prepared by *e.g.*, *de novo* synthesis of a portion of mouse monoclonal immunoglobulins comprising the desired portions (*e.g.*, antigen-binding region, CDR, FR, and/or C region) of murine origin can be produced using synthetic and/or recombinant nucleic acids to prepare constructs (*e.g.*, cDNA) encoding the desired monoclonal immunoglobulin chain. To prepare a portion of a chain, one or more stop codons can be introduced at the desired position. A sequence encoding a signal peptide can also be incorporated into the nucleic acid (*e.g.*, on synthesis, upon insertion into a vector). If the natural signal peptide sequence is unavailable, a signal peptide sequence from another antibody can be used (see, *e.g.*, Kettleborough, C.A., *Protein Engineering* 4:773-783 (1991)). Using these methods, methods described herein or other suitable methods, variants can be readily produced.

[0327] In one embodiment, a mouse monoclonal immunoglobulin of this invention comprises a light chain comprising SEQ ID NO: 3 and a heavy chain comprising SEQ ID NO: 16; a light chain comprising SEQ ID NO: 4 and a heavy chain comprising SEQ ID NO: 17; a light chain comprising SEQ ID NO: 5 and a heavy chain comprising SEQ ID NO: 18; a light chain comprising SEQ ID NO: 6 and a heavy chain comprising SEQ ID NO: 19; a light chain comprising SEQ ID NO: 7 and a heavy chain comprising SEQ ID NO: 20; a light chain comprising SEQ ID NO: 8 and a heavy chain comprising SEQ ID NO: 21; a light chain comprising SEQ ID NO: 9 and a heavy chain comprising SEQ ID NO: 22; a light chain comprising SEQ ID NO: 10 and a heavy chain comprising SEQ ID NO: 23; a light chain comprising SEQ ID NO: 11 and a heavy chain comprising SEQ ID NO: 24; a light chain comprising SEQ ID NO: 12 and a heavy chain comprising SEQ ID NO: 25; or a light chain comprising SEQ ID NO: 13 and a heavy chain comprising SEQ ID NO: 26.

[0328] In another embodiment, the invention also relates to a mouse monoclonal antibody that has binding specificity for human CD52, comprising a light chain variable region selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13; and a heavy chain variable region selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23; SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26.

[0329] If desired, for example, for diagnostic or assay purposes (*e.g.*, imaging), the mouse monoclonal immunoglobulin (*e.g.*, antigen binding fragment thereof) can comprise a detectable label. Suitable detectable labels and methods for labeling a mouse monoclonal immunoglobulin are well known in the art. Suitable detectable labels include, for example, a radioisotope (*e.g.*,
5 as Indium-111, Technetium-99m or Iodine-131), positron emitting labels (*e.g.*, Fluorine-19), paramagnetic ions (*e.g.*, Gadolinium (III), Manganese (II)), an epitope label (tag), an affinity label (*e.g.*, biotin, avidin), a spin label, an enzyme, a fluorescent group or a chemiluminescent group. When labels are not employed, complex formation (*e.g.*, between mouse monoclonal immunoglobulin and CD52) can be determined by surface plasmon resonance or other suitable
10 methods. All suitable methods and techniques described above for humanized antibodies of this invention can also be used herein.

Chimeric Immunoglobulins

[0330] As described herein, chimeric immunoglobulins having binding specificity for human CD52 have been produced. The chimeric immunoglobulin comprises a chimeric light chain
15 and/or a chimeric heavy chain that have amino acid sequences which differ from the amino acid sequence of known chimeric antibodies having binding specificity for human CD52.

[0331] As used herein, the term "chimeric immunoglobulin" refers to a recombinant protein that contains the variable domains including the complementarity determining regions (CDRs) of an antibody derived from one species, preferably a murine anti-human CD52 monoclonal
20 antibody, while the constant domains of the antibody molecule are derived from those of a different species, *e.g.*, from a human antibody.

[0332] The invention relates to the chimeric immunoglobulins described herein, including antigen-binding fragments (*i.e.*, portions) of the chimeric immunoglobulins, the chimeric light chains and chimeric heavy chains of the chimeric immunoglobulins and fragments of these
25 chimeric light and heavy chains. The invention relates to mature chimeric immunoglobulin, such as the chimeric immunoglobulin following processing to remove the heavy and light signal peptides and/or to the glycosylated immunoglobulin. The invention also relates to immature or precursor protein, such as a chimeric heavy chain comprising a signal peptide. The invention also relates to nucleic acid molecules (*e.g.*, vectors) that encode these immature or mature
30 proteins, to host cells that comprise such nucleic acids and to methods of producing these immature and mature proteins.

[0333] The binding function of a chimeric immunoglobulin having binding specificity for human CD52 can be detected using any suitable method, for example using assays which monitor formation of a complex between chimeric immunoglobulin and human CD52 (*e.g.*, a membrane fraction comprising human CD52, on a cell bearing human CD52, such as a human T cell, a human B cell, CHO cell or a recombinant host cell comprising a nucleic acid encoding human CD52, a peptide (*e.g.*, synthetic peptide) having an amino acid sequence of CD52).

[0334] Also provided herein are portions of the chimeric immunoglobulins which include light chains, heavy chains and portions of light and heavy chains. These immunoglobulin portions can be obtained or derived from immunoglobulins (*e.g.*, by reduction and/or cleavage), or nucleic acids encoding a portion of an immunoglobulin or chain thereof having the desired property (*e.g.*, binds human CD52, sequence similarity) can be produced and expressed. They may be prepared by *e.g.*, *de novo* synthesis of a portion. Chimeric immunoglobulins comprising the desired portions (*e.g.*, antigen-binding region, CDR, FR, and/or C region) of human and non-human origin can be produced using synthetic and/or recombinant nucleic acids to prepare constructs (*e.g.*, cDNA) encoding the desired chimeric chain. To prepare a portion of a chain, one or more stop codons can be introduced at the desired position. A sequence encoding a signal peptide can also be incorporated into the nucleic acid (*e.g.*, on synthesis, upon insertion into a vector). If the natural signal peptide sequence is unavailable (*e.g.*, typically not present), a signal peptide sequence from another antibody can be used (see, *e.g.*, Kettleborough, C.A., *Protein Engineering* 4:773-783 (1991)). Using these methods, methods described herein or other suitable methods, variants can be readily produced.

[0335] In one embodiment, a chimeric immunoglobulin of this invention comprises the light chain variable region of SEQ ID NO: 3 and the heavy chain variable region of SEQ ID NO: 16; the light chain variable region of SEQ ID NO: 4 and the heavy chain variable region of SEQ ID NO: 17; the light chain variable region of SEQ ID NO: 5 and the heavy chain variable region of SEQ ID NO: 18; the light chain variable region of SEQ ID NO: 6 and the heavy chain variable region of SEQ ID NO: 19; the light chain variable region of SEQ ID NO: 7 and the heavy chain variable region of SEQ ID NO: 20; the light chain variable region of SEQ ID NO: 8 and the heavy chain variable region of SEQ ID NO: 21; the light chain variable region of SEQ ID NO: 9 and the heavy chain variable region of SEQ ID NO: 22; the light chain variable region of SEQ ID NO: 10 and the heavy chain variable region of SEQ ID NO: 23; the light chain variable region of SEQ ID NO: 11 and the heavy chain variable region of SEQ ID NO: 24; the light chain

variable region of SEQ ID NO: 12 and the heavy chain variable region of SEQ ID NO: 25; or the light chain variable region of SEQ ID NO: 13 and the heavy chain variable region of SEQ ID NO: 26.

[0336] The invention also relates to a chimeric antibody that has binding specificity for human CD52, comprising a light chain variable region sequence selected from the group consisting of: the light chain variable region of SEQ ID NO: 3, the light chain variable region of SEQ ID NO: 4, the light chain variable region of SEQ ID NO: 5, the light chain variable region of SEQ ID NO: 6, the light chain variable region of SEQ ID NO: 7, the light chain variable region of SEQ ID NO: 8, the light chain variable region of SEQ ID NO: 9, the light chain variable region of SEQ ID NO: 10, the light chain variable region of SEQ ID NO: 11, the light chain variable region of SEQ ID NO: 12 and the light chain variable region of SEQ ID NO: 13, and a heavy chain variable region sequence selected from the group consisting of: the heavy chain variable region of SEQ ID NO: 16, the heavy chain variable region of SEQ ID NO: 17, the heavy chain variable region of SEQ ID NO: 18, the heavy chain variable region of SEQ ID NO: 19, the heavy chain variable region of SEQ ID NO: 20, the heavy chain variable region of SEQ ID NO: 21, the heavy chain variable region of SEQ ID NO: 22, the heavy chain variable region of SEQ ID NO: 23, the heavy chain variable region of SEQ ID NO: 24, the heavy chain variable region of SEQ ID NO: 25 and the heavy chain variable region of SEQ ID NO: 26.

[0337] The invention also relates to a chimeric light chain comprising the variable region of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or SEQ ID NO: 13.

[0338] The invention also relates to a chimeric heavy chain comprising the variable region of SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23; SEQ ID NO: 24, SEQ ID NO: 25 or SEQ ID NO: 26

[0339] If desired, for example, for diagnostic or assay purposes (*e.g.*, imaging), the chimeric immunoglobulin (*e.g.*, antigen-binding fragment thereof) can comprise a detectable label. Suitable detectable labels and methods for labeling a chimeric immunoglobulin are well known in the art. Suitable detectable labels include, for example, a radioisotope (*e.g.*, as Indium-111, Technetium-99m or Iodine-131), positron emitting labels (*e.g.*, Fluorine-19), paramagnetic ions (*e.g.*, Gadolinium (III), Manganese (II)), an epitope label (tag), an affinity label (*e.g.*, biotin, avidin), a spin label, an enzyme, a fluorescent group or a chemiluminescent group. When labels are not employed, complex formation (*e.g.*, between chimeric immunoglobulin and human

CD52) can be determined by surface plasmon resonance or other suitable methods. All suitable methods and techniques described above for humanized antibodies of this invention can also be used herein.

Nucleic Acids and Recombinant Vectors

- 5 [0340] The present invention also relates to isolated and/or recombinant (including, *e.g.*, essentially pure) nucleic acids comprising sequences which encode a humanized immunoglobulin, humanized light chain, humanized heavy chain, mouse monoclonal immunoglobulin, mouse immunoglobulin light chain, mouse immunoglobulin heavy chain, chimeric immunoglobulin, chimeric light chain or chimeric heavy chain of the present invention.
- 10 [0341] Nucleic acids referred to herein as “isolated” or “purified” are nucleic acids which have been separated away from the nucleic acids of the genomic DNA or cellular RNA of their source of origin (*e.g.*, as they exist in cells or in a mixture of nucleic acids such as a library), and include nucleic acids obtained by methods described herein or other suitable methods, including essentially pure nucleic acids, nucleic acids produced by chemical synthesis, by combinations of biological and
- 15 chemical methods, and recombinant nucleic acids which are isolated (see *e.g.*, Daugherty, B.L. *et al.*, *Nucleic Acids Res.*, 19(9): 2471-2476 (1991); Lewis, A.P. and J.S. Crowe, *Gene*, 101: 297-302 (1991)).
- [0342] Nucleic acids referred to herein as “recombinant” are nucleic acids which have been produced by recombinant DNA methodology, including those nucleic acids that are generated by procedures
- 20 which rely upon a method of artificial recombination, such as the polymerase chain reaction (PCR) and/or cloning into a vector using restriction enzymes. “Recombinant” nucleic acids are also those that result from recombination events that occur through the natural mechanisms of cells, but are selected for after the introduction to the cells of nucleic acids designed to allow and make probable a desired recombination event.
- 25 [0343] The present invention also relates more specifically to isolated and/or recombinant nucleic acids comprising a nucleotide sequence which encodes a humanized immunoglobulin, mouse immunoglobulin or chimeric immunoglobulin that has binding specificity for human CD52 (*e.g.*, a humanized immunoglobulin of the present invention in which the nonhuman portion (*e.g.*, the CDRs) is derived from a murine anti-CD52 monoclonal antibody; a mouse immunoglobulin of the
- 30 present invention; or a chimeric immunoglobulin of the present invention in which the nonhuman portion (*e.g.*, the V_H and V_L) is derived from a murine anti-CD52 monoclonal antibody) or portion (*e.g.*, antigen-binding portion) thereof (*e.g.*, heavy or light chain thereof).

[0344] Nucleic acids of the present invention can be used to produce humanized immunoglobulins having binding specificity for human CD52, mouse immunoglobulins having binding specificity for human CD52 and chimeric immunoglobulins having binding specificity for human CD52. For example, a nucleic acid (*e.g.*, DNA (such as cDNA), or RNA) or one or more nucleic acids encoding a humanized immunoglobulin, mouse immunoglobulin or chimeric immunoglobulin of the present invention can be incorporated into a suitable construct (*e.g.*, a recombinant vector) for further manipulation of sequences or for production of the encoded immunoglobulins in suitable host cells.

[0345] Constructs or vectors (*e.g.*, expression vectors) suitable for the expression of a humanized immunoglobulin having binding specificity for human CD52, mouse immunoglobulin having binding specificity for human CD52 or chimeric immunoglobulin having binding specificity for human CD52 are also provided. A variety of vectors are available, including vectors which are maintained in single copy or multiple copies in a host cell, or which become integrated into the host cell's chromosome(s). The constructs or vectors can be introduced into a suitable host cell, and cells which express a humanized immunoglobulin, mouse immunoglobulin or chimeric immunoglobulin of the present invention, can be produced and maintained in culture. A single vector or multiple vectors can be used for the expression of a humanized immunoglobulin, mouse immunoglobulin or chimeric immunoglobulin having binding specificity for human CD52.

[0346] Suitable expression vectors, for example mammalian cell expression vectors, can also contain a number of components, including, but not limited to one or more of the following: an origin of replication; a selectable marker gene; one or more expression control elements, such as a transcriptional control element (*e.g.*, a promoter, an enhancer, a terminator), and/or one or more translation signals; a signal sequence or leader sequence for membrane targeting or secretion. In a construct or vector, a signal peptide sequence can be provided by the construct or vector or other source. For example, the transcriptional and/or translational signals of an immunoglobulin can be used to direct expression.

[0347] A promoter can be provided for expression in a suitable host cell. Promoters can be constitutive or inducible. For example, a promoter can be operably linked to a nucleic acid encoding a humanized immunoglobulin or immunoglobulin chain, such that it directs expression of the encoded polypeptide. A variety of suitable promoters for prokaryotic (*e.g.*, lac, tac, T3, T7 promoters for *E. coli*) and eukaryotic (*e.g.*, yeast alcohol dehydrogenase (ADH1), SV40, CMV)

hosts are available. Those of skill in the art will be able to select the appropriate promoter for expressing an anti-CD52 antibody or portion thereof of the invention.

[0348] In addition, the vectors (*e.g.*, expression vectors) typically comprise a selectable marker for selection of host cells carrying the vector, and, in the case of a replicable vector, an origin of replication. Genes encoding products which confer antibiotic or drug resistance are common selectable markers and may be used in prokaryotic (*e.g.*, β -lactamase gene (ampicillin resistance), Tet gene (tetracycline resistance) and eukaryotic cells (*e.g.*, neomycin (G418 or geneticin), gpt (mycophenolic acid), ampicillin, or hygromycin resistance genes). Dihydrofolate reductase marker genes permit selection with methotrexate in a variety of hosts. Genes encoding the gene product of auxotrophic markers of the host (*e.g.*, *LEU2*, *URA3*, *HIS3*) are often used as selectable markers in yeast. Use of viral (*e.g.*, baculovirus) or phage vectors, and vectors which are capable of integrating into the genome of the host cell, such as retroviral vectors, are also contemplated.

[0349] The invention thus relates to isolated nucleic acid molecules that encode the humanized immunoglobulin, humanized light chain, humanized heavy chain, mouse immunoglobulin, mouse immunoglobulin light chain, mouse immunoglobulin heavy chain, chimeric immunoglobulin, chimeric light chain, or chimeric heavy chain of this invention. The invention also relates to isolated nucleic acid molecules that encode an antigen-binding portion of the immunoglobulins and their chains. Polypeptide sequences encoded by the nucleic acids of this invention are described above and in the following Examples.

[0350] In some embodiments, a nucleic acid and vector of this invention encodes a heavy chain (or an antigen-binding portion thereof) or a light chain (or an antigen-binding portion thereof) of this invention. A host cell containing both the heavy chain-encoding nucleic acid and the light chain-encoding nucleic acid can be used to make an antibody comprising the heavy and light chain (or an antigen-binding portion of the antibody). The heavy chain-encoding nucleic acid and the light chain-encoding nucleic acid can be placed on separate expression vectors. They can also be placed on a single expression vector under the same or different expression control. *See, e.g.*, Cabilly U.S. Patent 6,331,415; Fang U.S. Patent 7,662,623.

Method of Producing Immunoglobulins Having Specificity for Human CD52

[0351] Another aspect of the invention relates to a method of making an anti-human CD52 antibody of this invention. The antibody of this invention can be produced, for example, by the expression of one or more recombinant nucleic acids encoding the antibody in a suitable host cell. The host cell can be produced using any suitable method. For example, the expression constructs (*e.g.*, the one

- or more vectors, *e.g.*, a mammalian cell expression vector) described herein can be introduced into a suitable host cell, and the resulting cell can be maintained (*e.g.*, in culture, in an animal, in a plant) under conditions suitable for expression of the construct(s) or vector(s). Suitable host cells can be prokaryotic, including bacterial cells such as *E. coli* (*e.g.*, strain DH5 α TM (Invitrogen, Carlsbad, CA)), *B. subtilis* and/or other suitable bacteria; eukaryotic cells, such as fungal or yeast cells (*e.g.*, *Pichia pastoris*, *Aspergillus sp.*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Neurospora crassa*), or other lower eukaryotic cells, and cells of higher eukaryotes such as those from insects (*e.g.*, *Drosophila* Schnieder S2 cells, Sf9 insect cells (WO 94/26087 (O'Connor), TN5B1-4 (HIGH 5) insect cells (Invitrogen), mammals (*e.g.*, COS cells, such as COS-1 (ATCC Accession No. CRL-1650) and COS-7 (ATCC Accession No. CRL-1651), CHO (*e.g.*, ATCC Accession No. CRL-9096), CHO DG44 (Urlaub, G. and Chasin, LA., *Proc. Natl. Acad. Sci. USA*, 77(7):4216-4220 (1980)), 293 (ATCC Accession No. CRL-1573), HeLa (ATCC Accession No. CCL-2), CV1 (ATCC Accession No. CCL-70), WOP (Dailey, L., *et al.*, *J. Virol.*, 54:739-749 (1985)), 3T3, 293T (Pear, W. S., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 90:8392-8396 (1993)), NS0 cells, SP2/0 cells, HuT 78 cells and the like)), or plants (*e.g.*, tobacco, lemna (duckweed), and algae). (See, for example, Ausubel, F.M. *et al.*, eds. *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons Inc. (1993)). In some embodiments, the host cell is not part of a multicellular organism (*e.g.*, plant or animal), *e.g.*, it is an isolated host cell or is part of a cell culture.
- [0352] The present invention also relates to cells comprising a nucleic acid, *e.g.*, a vector, of the invention (*e.g.*, an expression vector). For example, a nucleic acid (*i.e.*, one or more nucleic acids) encoding the heavy and light chains of a humanized immunoglobulin, the heavy and light chains of mouse immunoglobulin, or the heavy and light chains of a chimeric immunoglobulin, said immunoglobulin having binding specificity for human CD52, or a construct (*i.e.*, one or more constructs, *e.g.*, one or more vectors) comprising such nucleic acid(s), can be introduced into a suitable host cell by a method appropriate to the host cell selected (*e.g.*, transformation, transfection, electroporation, infection), with the nucleic acid(s) being, or becoming, operably linked to one or more expression control elements (*e.g.*, in a vector, in a construct created by processes in the cell, integrated into the host cell genome). Host cells can be maintained under conditions suitable for expression (*e.g.*, in the presence of inducer, suitable media supplemented with appropriate salts, growth factors, antibiotic, nutritional supplements, etc.), whereby the encoded polypeptide(s) are produced. If desired, the encoded protein (*e.g.*, humanized

immunoglobulin, mouse immunoglobulin, chimeric immunoglobulin) can be isolated, for example, from the host cells, culture medium, or milk. This process encompasses expression in a host cell (e.g., a mammary gland cell) of a transgenic animal or plant (e.g., tobacco) (see e.g., WO 92/03918).

- 5 [0353] Fusion proteins can be produced in which an immunoglobulin portion (e.g., a humanized immunoglobulin; immunoglobulin chain) is linked to a non-immunoglobulin moiety (i.e., a moiety which does not occur in immunoglobulins as found in nature) in an N-terminal location, C-terminal location or internal to the fusion protein. For example, some embodiments can be produced by the insertion of a nucleic acid encoding an immunoglobulin sequence(s) into
- 10 a suitable expression vector, such as a pET vector (e.g., pET-15b, Novagen), a phage vector (e.g., pCANTAB 5 E, Pharmacia), or other vector (e.g., pRIT2T Protein A fusion vector, Pharmacia). The resulting construct can be introduced into a suitable host cell for expression. Upon expression, some fusion proteins can be isolated or purified from a cell lysate by means of a suitable affinity matrix (see, e.g., *Current Protocols in Molecular Biology* (Ausubel, F.M.
- 15 *et al.*, Eds., Vol. 2, Suppl. 26, pp. 16.4.1-16.7.8 (1991)).

- [0354] The invention relates to a host cell that comprises recombinant nucleic acid(s) encoding an immunoglobulin provided herein (e.g., a humanized immunoglobulin, a humanized light chain or a humanized heavy chain, a mouse immunoglobulin, a mouse light chain or a mouse heavy chain, a chimeric immunoglobulin, a chimeric heavy chain, or a chimeric light chain of
- 20 the invention). The invention also relates to a host cell that comprises recombinant nucleic acid(s) encoding an antigen-binding portion of the immunoglobulin or their chains. In some embodiments, the host cell comprises a recombinant vector (e.g., expression vector, mammalian cell expression vector) of the invention as referred to herein.

- [0355] The invention also relates to a method of preparing an immunoglobulin or an
- 25 immunoglobulin polypeptide chain of this invention. In one embodiment, the method comprises maintaining a host cell of the invention as described herein (e.g., a host cell that contains one or more isolated nucleic acids that encode the immunoglobulin or polypeptide chain (e.g., a light chain and a heavy chain, a light chain only, or a heavy chain only, of the invention) under conditions appropriate for expression of the immunoglobulin or polypeptide chain. For example
- 30 a host cell can be cultured on a substrate or in suspension. In some embodiments, the method further comprises the step of purifying or isolating the immunoglobulin or polypeptide chain.

- [0356] The invention further relates to a method of preparing immunoglobulins through phage display. For example, a naïve antibody phage display library on CD52 antigen can be panned. Alternatively, a method of preparing immunoglobulins through guided selection can be used (U.S. Patent Application Publication US 2006-0251658 A1.) A custom library built around, for example, a fixed heavy chain (and/or light chain) CDR3 region of a known anti-CD52 antibody can be created. The CDR1 and CDR2 regions of the heavy and light chains can be derived from a naïve repertoire (Osburn *et al.*, *Methods*, 36:61-68 (2005)). In one embodiment, anti-CD52 ScFvs can be generated from ScFv naïve antibody libraries which are used to obtain mouse-human chimeric antibodies with the desired binding properties. These libraries may be screened for antibodies with the desired binding properties. ScFv phage libraries may be used. For example, ScFvs which recognize human CD52 can be isolated from scFv guided selection libraries following a series of repeated selection cycles on recombinant human CD52 essentially as described in Vaughan *et al.* (1996). In brief, following incubation with the library, the immobilized antigen, which is pre-coupled to paramagnetic beads, and bound phage can be recovered by magnetic separation while unbound phage is washed away. Bound phage can then be rescued as described by Vaughan *et al.* (1996) and the selection process repeated.
- [0357] In a particular embodiment, a library is constructed consisting of the entire variable domain of the heavy chain of a mouse anti-CD52 antibody fused in a single chain format to a repertoire of naïve human light chain variable regions. After selection the pool of human light chain variable regions that complement the mouse heavy chain variable region are identified. A library is then constructed consisting of the repertoire of human light chain variable regions selected above fused in a single chain format to a chimeric heavy chain variable region consisting of naïve human CDR1 and CDR2 regions and a fixed CDR3 region from the mouse anti-CD52 antibody heavy chain variable domain. After selection for CD52 binders, the best binding clones are selected. Five of the 6 CDR regions can be human in origin while the CDR-3 of the heavy chain variable region can be identical to the original CDR3 of the mouse heavy chain variable domain.
- [0358] Selections can be performed using CD52 coupled to DYNABEADS M-270 amine (Dyna) according to the manufacturer's recommendations. Alternatively, selections using biotinylated CD52 can be prepared using the primary amine specific reagent succinimidyl-6-(biotinamido) hexanoate following the manufacturer's instructions (EZ link NHS LC Biotin, Pierce).

[0359] Outputs from selections can be tested as periplasmic preparations in high throughput screens based on competition assays which measure the ability of the scFvs present in the periplasmic preparation to compete for binding to CD52.

[0360] Samples that are able to compete in the high throughput screens may be subjected to
5 DNA sequencing as described in Vaughan *et al.* (1996) and Osburn *et al.* (1996). Clones would then be expressed and purified as scFvs or IgGs and assessed for their ability to bind CD52, neutralize CD52 or a combination thereof, *e.g.*, using assays such as antibody-dependent cell mediated cytotoxicity (ADCC) assay and complement dependent cytotoxicity (CDC) assay. Purified scFv preparations can then be prepared as described in Example 3 of WO 01/66754.
10 Protein concentrations of purified scFv preparations were determined using the BCA method (Pierce). Similar approaches can be used to screen for an optimal partner (the opposite chain) of a fixed immunoglobulin heavy or light chain (or V_H or V_L).

[0361] In a particular embodiment, the invention is directed to a method of producing a hybridoma that secretes a monoclonal antibody that has binding specificity for human CD52
15 comprising administering lymphocytes of a CD52 transgenic mouse to a non-transgenic mouse having the same strain (*e.g.*, CD1) as the human CD52 transgenic mouse, thereby producing an immunized, non-transgenic mouse. Splenocytes of the immunized, non-transgenic mouse are contacted with immortalized cells, thereby producing fused cells, and the fused cells are maintained under conditions in which hybridomas that secrete a monoclonal antibody having
20 binding specificity for human CD52 are produced, thereby producing a hybridoma that secretes a monoclonal antibody that has binding specificity for human CD52.

Immunoglobulins Containing a Toxin Moiety or Toxin

[0362] The invention also relates to immunoglobulins that comprise a toxin moiety or toxin. Suitable toxin moieties comprise a toxin (*e.g.*, surface active toxin, cytotoxin). The toxin moiety
25 or toxin can be linked or conjugated to the immunoglobulin using any suitable method. For example, the toxin moiety or toxin can be covalently bonded to the immunoglobulin directly or through a suitable linker. Suitable linkers can include noncleavable or cleavable linkers, for example, pH cleavable linkers or linkers that comprise a cleavage site for a cellular enzyme (*e.g.*, cellular esterases, cellular proteases such as cathepsin B). Such cleavable linkers can be
30 used to prepare an immunoglobulin that can release a toxin moiety or toxin after the immunoglobulin is internalized.

[0363] A variety of methods for linking or conjugating a toxin moiety or toxin to an immunoglobulin can be used. The particular method selected will depend on the toxin moiety or toxin and immunoglobulin to be linked or conjugated. If desired, linkers that contain terminal functional groups can be used to link the immunoglobulin and toxin moiety or toxin. Generally, conjugation is accomplished by reacting toxin moiety or toxin that contains a reactive functional group (or is modified to contain a reactive functional group) with a linker or directly with an immunoglobulin. Covalent bonds are formed by reacting a toxin moiety or toxin that contains (or is modified to contain) a chemical moiety or functional group that can, under appropriate conditions, react with a second chemical group thereby forming a covalent bond. If desired, a suitable reactive chemical group can be added to an immunoglobulin or to a linker using any suitable method. (See, e.g., Hermanson, G. T., *Bioconjugate Techniques*, Academic Press: San Diego, CA (1996).) Many suitable reactive chemical group combinations are known in the art, for example an amine group can react with an electrophilic group such as tosylate, mesylate, halo (chloro, bromo, fluoro, iodo), N-hydroxysuccinimidyl ester (NHS), and the like. Thiols can react with maleimide, iodoacetyl, acryloyl, pyridyl disulfides, 5-thiol-2-nitrobenzoic acid thiol (TNB-thiol), and the like. An aldehyde functional group can be coupled to amine- or hydrazide-containing molecules, and an azide group can react with a trivalent phosphorous group to form phosphoramidate or phosphorimide linkages. Suitable methods to introduce activating groups into molecules are known in the art (see for example, Hermanson, G. T., *Bioconjugate Techniques*, Academic Press: San Diego, CA (1996)).

[0364] Suitable toxin moieties and toxins include, for example, a maytansinoid (e.g., maytansinol, e.g., DM1, DM4), a taxane, a calicheamicin, a duocarmycin, or derivatives thereof. The maytansinoid can be, for example, maytansinol or a maytansinol analogue. Examples of maytansinol analogs include those having a modified aromatic ring (e.g., C-19-dechloro, C-20-demethoxy, C-20-acyloxy) and those having modifications at other positions (e.g., C-9-CH₃, C-14-alkoxymethyl, C-14-hydroxymethyl or acetoxy, C-15-hydroxy/acyloxy, C-15-methoxy, C-18-N-demethyl, 4,5-deoxy). Maytansinol and maytansinol analogs are described, for example, in U.S. Patent Nos 5,208,020 and 6,333,410, the contents of which are incorporated herein by reference. Maytansinol can be coupled to antibodies and antibody fragments using, e.g., an N-succinimidyl 3-(2-pyridyldithio)propionate (also known as N-succinimidyl 4-(2-pyridyldithio)pentanoate (or SPP)), 4-succinimidyl-oxycarbonyl- α -(2-pyridyldithio)-toluene (SMPT), N-succinimidyl-3-(2-pyridyldithio)butyrate (SDPB), 2 iminothiolane, or S-

acetylsuccinic anhydride. The taxane can be, for example, a taxol, taxotere, or novel taxane (see, *e.g.*, WO 01/38318). The calicheamicin can be, for example, a bromo-complex calicheamicin (*e.g.*, an alpha, beta or gamma bromo-complex), an iodo-complex calicheamicin (*e.g.*, an alpha, beta or gamma iodo-complex), or analogs and mimics thereof. Bromo-complex calicheamicins include I1-BR, I2-BR, I3-BR, I4-BR, J1-BR, J2-BR and K1-BR. Iodo-complex calicheamicins include I1-I, I2-I, I3-I, J1-I, J2-I, L1-I and K1-BR. Calicheamicin and mutants, analogs and mimics thereof are described, for example, in U.S. Patent Nos 4,970,198; 5,264,586; 5,550,246; 5,712,374, and 5,714,586, the contents of each of which are incorporated herein by reference. Duocarmycin analogs (*e.g.*, KW-2189, DC88, DC89 CBI-TMI, and derivatives thereof) are described, for example, in U.S. Patent No. 5,070,092, U.S. Patent No. 5,187,186, U.S. Patent No. 5,641,780, U.S. Patent No. 5,641,780, U.S. Patent No. 4,923,990, and U.S. Patent No. 5,101,038, the contents of each of which are incorporated herein by reference.

[0365] Examples of other toxins include, but are not limited to antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, CC-1065 (see US Patent Nos. 5,475,092, 5,585,499, 5,846,545), melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, mitomycin, puromycin anthramycin (AMC)), duocarmycin and analogs or derivatives thereof, and anti-mitotic agents (*e.g.*, vincristine, vinblastine, taxol, auristatins (*e.g.*, auristatin E) and maytansinoids, and analogs or homologs thereof).

[0366] The toxin can also be a surface active toxin, such as a toxin that is a free radical generator (*e.g.*, selenium containing toxin moieties), or radionuclide containing moiety. Suitable radionuclide containing moieties, include for example, moieties that contain radioactive iodine (131I or 125I), yttrium (90Y), lutetium (177Lu), actinium (225Ac), praseodymium, astatine (211At), rhenium (186Re), bismuth (212Bi or 213Bi), indium (111In), technetium (99mTc), phosphorus (32P), rhodium (188Rh), sulfur (35S), carbon (14C), tritium (3H), chromium (51Cr), chlorine (36Cl), cobalt (57Co or 58Co), iron (59Fe), selenium (75Se), or gallium (67Ga).

[0367] The toxin can be a protein, polypeptide or peptide, from bacterial sources, *e.g.*, diphtheria toxin, pseudomonas exotoxin (PE) and plant proteins, *e.g.*, the A chain of ricin

(RTA), the ribosome inactivating proteins (RIPs) gelonin, pokeweed antiviral protein, saporin, and dodecandron are contemplated for use as toxins.

[0368] Antisense compounds of nucleic acids designed to bind, disable, promote degradation or prevent the production of the mRNA responsible for generating a particular target protein can also be used as a toxin. Antisense compounds include antisense RNA or DNA, single or double stranded, oligonucleotides, or their analogs, which can hybridize specifically to individual mRNA species and prevent transcription and/or RNA processing of the mRNA species and/or translation of the encoded polypeptide and thereby effect a reduction in the amount of the respective encoded polypeptide. Ching, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 86: 10006-10010 (1989); Broder, *et al.*, *Ann. Int. Med.* 113: 604-618 (1990); Loreau, *et al.*, *FEBS Letters* 274: 53-56 (1990). Useful antisense therapeutics include for example: Veglin TM (VasGene) and OGX-011 (Oncogenix).

[0369] Toxins can also be photoactive agents. Suitable photoactive agents include porphyrin-based materials such as porfimer sodium, the green porphyrins, chlorin E6, hematoporphyrin derivative itself, phthalocyanines, etiopurpurins, texaphrin, and the like.

[0370] The toxin can be an antibody or antibody fragment that binds an intracellular target. Such antibodies or antibody fragments can be directed to defined subcellular compartments or targets. For example, the antibodies or antibody fragments can bind an intracellular target selected from erbB2, EGFR, BCR-ABL, p21Ras, Caspase3, Caspase7, Bcl-2, p53, Cyclin E, ATF-1/CREB, HPV16 E7, HP1, Type IV collagenases, cathepsin L as well as others described in Kontermann, R.E., *Methods*, 34:163-170 (2004), incorporated herein by reference in its entirety.

Therapeutic Methods and Compositions

[0371] The antibodies of this invention are useful in immuno-suppression and immuno-ablation. The antibodies target CD52-expressing cells (e.g., T and B cells) and reduce (or “deplete” as used herein) their population in a subject in need thereof. Lymphocyte depletion may be useful in treating a variety of diseases and conditions such as inflammation, autoimmune diseases and cancer (e.g., lymphocyte (either B or T cell) malignancy). See, e.g., Reiff, A., *Hematology*, 10(2):79-93 (2005). Examples of diseases and conditions that can be treated with the antibodies or antigen-binding portions of this invention include, without limitation, multiple sclerosis, lupus, rheumatoid arthritis, graft versus host disease (GVHD), inflammatory bowel disease, vasculitis, Behcet’s disease, Wegener’s granulomatosis, Sjogren’s syndrome, uveitis,

psoriasis, scleroderma, polymyositis, type I (autoimmune-based) diabetes, autoimmune cytopenias (e.g., autoimmune neutropenia, transfusion-dependent refractory PRCA, leukemia and lymphoma such as non-Hodgkin's lymphoma with bulky disease and B-cell chronic lymphocytic leukemia.

- 5 [0372] Accordingly, aspects of this invention are methods for lymphocyte depletion, and for treating inflammation, an autoimmune disease or cancer by administering an effective amount of an antibody of the invention to a subject in need thereof (e.g., a human patient having an autoimmune disease, a blood cancer, or a patient who is to receive a transplantation). The antibody also can be administered prophylactically to prevent onset of inflammation or relapse
- 10 of an autoimmune disease or cancer. For example, the antibody of this invention can be administered as part of a conditioning regimen to prepare a patient for a transplantation (e.g., a stem cell transplant, an infusion of autologous or allogeneic T cells, or a solid organ transplant).
- [0373] Some anti-CD52 antibodies of this invention preferentially target certain populations of CD52+ cells. One possible explanation is that epitopes to which these antibodies bind include
- 15 one or more carbohydrate groups on the CD52 protein, and such carbohydrate groups may be more prevalent on CD52 expressed on one cell type versus another. For example, we have found that antibody 7F11, 5F7, 3G7, and 11C11 deplete T cells to a greater extent than B cells. Thus, the humanized and chimeric versions of these antibodies may be used to treat T cell malignancy with milder immunosuppressing side effects.
- 20 [0374] Because antibodies of this invention target CD52-expressing cells, the antibodies also can be used to deplete CD52+ cell types other than T cells and B cells. For example, studies have shown that vascular leukocytes (VLC) and Tie2+ monocytes – myeloid cells expressing high levels of CD52 – promote tumor angiogenesis and contribute to tumor resistance to anti-VEGF therapy. Pulaski et al., *J. Translational Med.* 7:49 (2009). Anti-CD52 antibodies of this
- 25 invention thus can be used to inhibit tumor angiogenesis by targeting VLC and Tie2+ monocytes. For this purpose, the anti-CD52 antibodies can be administered systemically, or locally at a site of neovascularization, such as a tumor site. Anti-CD52 antibody therapy can be used in conjunction with standard-of-care cancer treatment such as chemotherapy, surgery, or radiation, or with another targeted therapy such as anti-VEGF antibody therapy. Anti-CD52
- 30 antibody therapy can be used to treat, for example, breast cancer, lung cancer, glioma, colorectal cancer, and any other indications of anti-VEGF antibodies. Anti-CD52 antibody therapy also

can be used in other neovascularization conditions including non-oncological neovascular conditions.

[0375] Antibodies of this invention can be administered to an individual (e.g., a human) alone or in conjunction with another agent (e.g., an immunosuppressant) in a combination therapy.

- 5 The antibody can be administered before, along with or subsequent to administration of the additional agent. In some embodiments, the additional agent is, for example, an anti-inflammatory compound such as sulfasalazine, another non-steroidal anti-inflammatory compound, or a steroidal anti-inflammatory compound. In some embodiments, the additional agent is another lympho-depleting antibody such as another anti-CD52 antibody, an anti-CD20 antibody, an anti-BAFF antibody, an anti-BAFF-R antibody, and the like. In some embodiments, the additional agent is, e.g., a cytokine (e.g., IL-7), anti-cytokine receptor antibody, or a soluble receptor, that skews, manipulates, and/or augments the reconstitution process that occurs following lymphodepletion mediated by an anti-CD52 antibody (see, e.g., Sportes et al., "Cytokine Therapies: *Ann. N.Y. Acad. Sci.* 1182:28-38 (2009)). In another embodiment, a synthetic peptide mimetic can be administered in
- 15 conjunction with an immunoglobulin of the present invention.

[0376] Studies have shown that lymphocyte depletion by alemtuzumab is mediated by neutrophils and NK cells (Hu et al., *Immunology* 128:260-270 (2009)). Thus, in an embodiment of combination therapy, an agent that stimulates neutrophils and NK cells can be administered to a patient, before, during or after anti-CD52 antibody therapy, to augment the antibody therapy.

- 20 Stimulating neutrophils and/or NK cells include, without limitation, (1) increasing their rates of division, (2) increasing their cell surface expression of the Fc receptors corresponding to the isotype of the anti-CD52 antibody (e.g., FcγRIIIa and FcγRIIIb, FcγRII, FcγRI, and FcαRI), (3) mobilizing and increasing the number of circulating cells, (4) recruiting the cells to target sites (e.g., sites of tumors, inflammation, or tissue damage), (5) and increasing their cytotoxic
- 25 activity. Examples of agents that stimulate neutrophils and/or NK cells include, for example, granulocyte monocyte colony stimulating factor (GM-CSF) (e.g., LEUKINE® or sargramostim and molgramostim); granulocyte colony stimulating factor (G-CSF) (e.g., NEUPOGEN® or filgrastim, pegylated filgrastim, and lenograstim); interferon gamma (e.g., ACTIMMUNE®); CXC chemokine receptor 4 (CXCR4) antagonists (e.g., MOZOBIL™ or plerixafor); and CXC
- 30 chemokine receptor 2 (CXCR2) agonists. The neutrophil count of the patient may be monitored periodically to ensure optimal treatment efficacy. The neutrophil count of the patient also can be measured prior to the start of the anti-CD52 antibody treatment. The stimulator's amount can be

adjusted based on the patient's neutrophil count. A higher dose of the stimulator may be used if the patient has a lower than normal neutrophil count. During periods of neutropenia, which may be caused by treatment with the anti-CD52 antibody, a higher dose of the neutrophil stimulator may also be administered to maximize the effect of the anti-CD52 antibody.

5 [0377] Because neutrophil and/or NK stimulation improves the efficacy of anti-CD52 antibody therapy, this embodiment of combination therapy allows one to use less antibody in a patient while maintaining similar treatment efficacy. Using less anti-CD52 antibody while maintaining treatment efficacy may help reduce side effects of the anti-CD52 antibody, which include immune response in the patient against the administered antibody as well as development of
10 secondary autoimmunity (autoimmunity that arises during or after anti-CD52 antibody treatment). This embodiment of combination of therapy is also useful in an oncology setting, e.g., when the patient has neutropenia.

[0378] In another embodiment of combination therapy, one can use a stimulator of regulatory T cells to augment anti-CD52 antibody therapy. Our data show that anti-CD52 antibodies deplete
15 CD4⁺CD25⁺FoxP3⁺ regulatory T cells to a much lesser extent as compared to other CD4⁺ T cells. Regulatory T cells (also known as "Treg" or suppressor T cells) are cells that are capable of inhibiting the proliferation and/or function of other lymphoid cells via contact-dependent or contact-independent (e.g., cytokine production) mechanisms. Several types of regulatory T cells have been described, including $\gamma\delta$ T cells, natural killer T (NKT) cells, CD8⁺T cells, CD4⁺T
20 cells, and double negative CD4⁺CD8⁺T cells. See, e.g., Bach et al., *Immunol.* 3:189-98 (2003). CD4⁺CD25⁺FoxP3⁺ regulatory T cells have been referred as "naturally occurring" regulatory T cells; they express CD4, CD25 and forkhead family transcription factor FoxP3 (forkhead box p3). Thus, in this embodiment of combination therapy, one can administer an agent that stimulates CD4⁺CD25⁺FoxP3⁺ regulatory T cells before, during or after the anti-CD52 antibody
25 therapy, to skew the composition of the immune system following lympho-depletion. The agent may, for example, activate those T cells, stabilize and/or expand the population of the cells, mobilize and increase circulation of the cells, and/or recruit the cells to target sites. Examples of such agents are rapamycin, active or latent TGF- β (e.g., TGF- β 1, TGF- β 2, TGF- β 3, TGF- β 4, and TGF- β 5), IL-10, IL-4, IFN- α , vitamin D3, dexamethasone, and mycophenolate mofetil (see,
30 e.g., Barrat et al., *J. Exp. Med.* **195**:603-616 (2002); Gregori et al., *J Immunol.* **167**: 1945-1953 (2001); Battaglia et al., *Blood* **105**: 4743-4748 (2005); Battaglia et al., *J. Immunol.* 177:8338-8347 (2006)).

[0379] In this invention, an effective amount of anti-CD52 antibody for treating a disease is an amount that helps the treated subject to reach one or more desired clinical end points. For example, for lupus (whose manifestations include systemic lupus erythematosus, lupus nephritis, cutaneous lupus erythematosus, CNS lupus, cardiovascular manifestations, pulmonary
5 manifestations, hepatic manifestations, haematological manifestations, gastrointestinal manifestations, musculoskeletal manifestations, neonatal lupus erythematosus, childhood systemic lupus erythematosus, drug-induced lupus erythematosus, anti-phospholipid syndrome, and complement deficiency syndromes resulting in lupus manifestations; see, e.g., Robert G. Lahita, Editor, *Systemic Lupus Erythematosus*, 4th Ed., Elsevier Academic Press, 2004), clinical
10 endpoints can be measured by monitoring of an affected organ system (e.g., hematuria and/or proteinuria for lupus nephritis) and/or using a disease activity index that provides a composite score of disease severity across several organ systems (e.g., BILAG, SLAM, SLEDAI, ECLAM). See, e.g., Mandl et al., "Monitoring patients with systemic lupus erythematosus" in *Systemic Lupus Erythematosus*, 4th edition, pp. 619-631, R.G. Lahita, Editor, Elsevier Academic
15 Press, (2004).

[0380] In another example of autoimmune disease, multiple sclerosis (including relapsing-remitting, secondary progressive, primary progressive, and progressive relapsing multiple sclerosis ((Lublin et al., *Neurology* **46** (4), 907-11 (1996)), diagnosed is made by, for example, the history of symptoms and neurological examination with the help of tests such as magnetic
20 resonance imaging (MRI), spinal taps, evoked potential tests, and laboratory analysis of blood samples. In MS, the goal of treatment is to reduce the frequency and severity of relapses, prevent disability arising from disease progression, and promote tissue repair (Compston and Coles, 2008). Thus, an amount of anti-CD52 antibody that helps achieve a clinical endpoint consistent with that goal is an effective amount of antibody for the treatment.

25 [0381] To minimize immunogenicity, it is preferred that a humanized antibody be used to treat a human patient in therapeutic methods and compositions of this invention. In cases where repeated administration is not necessary, it may also be appropriate to administer a mouse:human chimeric antibody of this invention to a human patient.

[0382] The antibodies of the invention can be used to treat an individual who has previously
30 been treated with Campath-1H® who has developed neutralizing antibodies to Campath-1H® (e.g., a Campath-1H®-refractory individual). For example, one could treat an individual having an autoimmune disease (e.g., multiple sclerosis, lupus, vasculitis) and/or a cancer (e.g., a

leukemia (e.g., chronic lymphocytic leukemia), a lymphoma (e.g., non-Hodgkin's lymphoma)) who has previously been treated with Campath-1H® (e.g., with one or more courses of Campath-1H® treatment) and who has developed neutralizing antibodies to Campath-1H® that reduce the efficacy of further Campath-1H® treatment. We have shown that the humanized
5 antibodies of this invention (e.g., humanized 2C3, 12G6, and 9D9) can bind to human CD52 despite the presence of neutralizing antibodies to alemtuzumab. In another embodiment, one could treat an individual who had become refractory to treatment with a particular humanized antibody described herein with one of the other humanized antibodies described herein.

[0383] The antibody of this invention can be administered in a single unit dose or multiple doses
10 at any time point deemed appropriate by a health care provider. The dosage can be determined by methods known in the art and can be dependent, for example, upon the individual's age, sensitivity, tolerance and overall well-being. A variety of routes of administration can be used, including, but not necessarily limited to, parenteral (e.g., intravenous, intraarterial, intramuscular, intrathecal, intraperitoneal, subcutaneous injection), oral (e.g., dietary), locally, topical, inhalation (e.g.,
15 intrabronchial, intranasal or oral inhalation, intranasal drops), or rectal, depending on the disease or condition to be treated. Parenteral administration may be one preferred mode of administration.

[0384] In one embodiment, the antibodies of the invention are administered to a patient using the same dosing regimen as Campath-1H® (e.g., the dosing regimen of Campath-1H® for chronic lymphocytic leukemia). In another embodiment, an antibody of the invention is
20 administered to a patient having an autoimmune disease (e.g., multiple sclerosis (MS)) in a regimen comprising administration of a first cycle of the antibody followed by at least one further cycle of the antibody, in which each treatment cycle comprises 1-5 doses that are applied on consecutive days, and wherein each treatment cycle is separated from the next cycle by at least 1 - 24 months (e.g., 12 months). For example, in one embodiment, a patient having
25 multiple sclerosis is treated with a first cycle of the antibody comprising 5 daily doses of the antibody followed by at least one further cycle of antibody treatment, in which the treatment occurs 12 months after the first cycle and comprises 3 doses of the antibody applied on consecutive days. In another embodiment, a patients having MS is only re-treated once evidence of renewed MS activity has been observed (see, e.g., WO 2008/031626; the teachings of which
30 are incorporated herein by reference in their entirety). In some embodiments, it may be necessary to administer more frequent courses of treatment (e.g., every four months, every six months) if patients with more advanced forms of MS or more progressive forms of other

autoimmune diseases (such as vasculitis; *see, e.g.,* Walsh et al., *Ann Rheum Dis* 67:1322-1327 (2008)) experience a relapse early on after their last course of treatment. Evidence of renewed MS activity may be determined based on the professional judgment of the treating clinician, using any means that may be available to such clinician. A variety of techniques are currently available to clinicians to diagnose renewed MS activity including, without limitation, by clinical means (relapse or progression of neurological disability) or by magnetic resonance imaging (MRI) of the brain or spinal cord. As is well understood by medical practitioners, disease activity detected via MRI may be indicated by the occurrence of new cerebral or spinal lesions on T1 (enhanced or non-enhanced)- or T2-weighted images or by the increase of the volume of such lesions. As diagnostic methods for MS are continually evolving, it is anticipated there may be additional methods in the future that will detect renewed MS activity (e.g., magnetization transfer ratio or MR-spectroscopy). The particular diagnostic method used to detect renewed MS activity is not a limitation of the claimed invention. In certain embodiments, repeated MRIs are performed in fixed intervals after a treatment cycle in order to determine whether re-treatment of any given patient is necessary and the optimal time point for re-treatment of such patient. In general, it is desirable for re-treatment to occur before the disease re-manifests clinically.

[0385] Formulation will vary according to the route of administration selected (*e.g.,* solution, emulsion). An appropriate composition comprising the antibody to be administered can be prepared in a physiologically acceptable vehicle or carrier. The composition can comprise multiple doses or be a single unit dose composition. For solutions or emulsions, suitable carriers include, for example, aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles can include various additives, preservatives, or fluid, nutrient or electrolyte replenishers (See, generally, *Remington's Pharmaceutical Sciences*, 17th Edition, Mack Publishing Co., PA, 1985). For inhalation, the compound can be solubilized and loaded into a suitable dispenser for administration (*e.g.,* an atomizer, nebulizer or pressurized aerosol dispenser).

Diagnostic Methods and Compositions

[0386] The immunoglobulins of the present invention also are useful in a variety of processes with applications in research and diagnosis. For instance, they can be used to detect, isolate, and/or purify human CD52 or variants thereof (*e.g.,* by affinity purification or other suitable methods

such as flow cytometry, *e.g.*, for cells, such as lymphocytes, in suspension), and to study human CD52 structure (*e.g.*, conformation) and function. For *in vitro* applications, wherein immunogenicity of the antibody is not a concern, the mouse and chimeric antibodies of this invention will be useful in addition to humanized antibodies.

- 5 [0387] The immunoglobulins of the present invention can be used in diagnostic applications (*e.g.*, *in vitro*, *ex vivo*). For example, the humanized immunoglobulins of the present invention can be used to detect and/or measure the level of human CD52 in a sample (*e.g.*, on cells expressing human CD52 in tissues or body fluids, such as an inflammatory exudate, blood, serum, bowel fluid, tissues bearing human CD52). A sample (*e.g.*, tissue and/or body fluid) can be obtained from an individual and an immunoglobulin described herein can be used in a suitable immunological method to detect and/or measure human CD52 expression, including methods such as flow cytometry (*e.g.*, for cells in suspension such as lymphocytes), enzyme-linked immunosorbent assays (ELISA), including chemiluminescence assays, radioimmunoassay, and immunohistology.
- 10 [0388] In one embodiment, a method of detecting human CD52 in a sample is provided, comprising contacting a sample with an immunoglobulin of the present invention under conditions suitable for specific binding of the immunoglobulin to human CD52 and detecting antibody-CD52 complexes which are formed. In an application of the method, the immunoglobulins described herein can be used to analyze normal versus inflamed tissues (*e.g.*, from a human) for human CD52 reactivity and/or expression (*e.g.*, immunohistologically) to detect associations between *e.g.*, inflammatory bowel disease (IBD), autoimmune diseases (such as multiple sclerosis and lupus), cancer (such as non-Hodgkin's lymphoma and chronic lymphocytic leukemia), or other conditions and increased expression of human CD52 (*e.g.*, in affected tissues). Thus, the immunoglobulins of the present invention permit immunological methods of assessment of the presence of human CD52 in normal and inflamed tissues, through which the presence of disease, disease progress and/or the efficacy of anti-human CD52 therapy in the treatment of disease, *e.g.*, inflammatory disease can be assessed.
- 15 [0389] In addition, the immunoglobulins can be used to examine tissues after treatment with a depleting anti-CD52 therapeutic antibody to gauge how effective the depletion has been as well as to determine whether there has been any downregulation in the expression of CD52 (Rawstrom *et al.*, *Br. J. Haem.*, 107:148-153 (1999)).
- 20 [0390] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention

belongs. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention. All publications and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. Although a number of documents are cited herein, this citation does not constitute an admission that any of these documents forms part of the common general knowledge in the art. Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. The materials, methods, and examples are illustrative only and not intended to be limiting.

EXEMPLIFICATION

Example 1: Generation of Mouse Anti-Human CD52 Antibodies

[0391] The mouse anti-human CD52 antibodies in the following working examples were generated by immunizing CD1 strain mice with splenocytes from human CD52 transgenic mice with a CD1 background (FIG. 1A), where display of human CD52 on the surface of mouse B and T cells of the transgenic mice was verified by flow cytometry. Because the transgenic mice had the same background (CD1) as the immunized mice, splenocytes from the transgenic mice presented human CD52 at the cell surface as a unique, non-self antigen in a native format, and the immunized nontransgenic mice mounted an antibody response primarily towards the human CD52.

[0392] To collect splenocytes from the human CD52 transgenic mice, the mice were euthanized, spleens were harvested and single cell suspensions were prepared by passing through a syringe. CD1 mice were then immunized with the collected human CD52 positive spleen cells at 5×10^6 in 100 μ l per mouse with or without Freund's Complete Adjuvant by intraperitoneal (i.p.) injection. Mice were given two booster doses every two weeks after first immunization with transgenic mouse human CD52 positive spleen cells at 5×10^6 in 100 μ l per mouse with Freund's Incomplete Adjuvant, ip.

[0393] Eye bleeds were collected 100-200 μ l per mouse in yellow-capped serum separator tubes from all mice before immunization to determine base level reactivity, and a week after every round of immunization to determine base level reactivity, and a week after every round of immunization to determine anti-human CD52 specific immune response. Mice that mounted

high levels of anti-human CD52 reactivity as measured by FACS on CHO K1 cells engineered to express human CD52 protein, but not on parental CHO K1 cells were sacrificed, blood was harvested and spleens were collected under sterile conditions to generate hybridomas.

Hybridomas were generated by using a non-secreting mouse myeloma cell-line SP2/0 Ag14 or

- 5 NS1 myeloma cells as fusion partners 3-4 days post immunization. Fused cells were placed in complete growth medium containing hypoxanthine, aminopterin and thymidine to generate hybridomas. After screening many hybridoma supernatants, several clones were selected that produced specific anti-human CD52 antibodies and were further subcloned to derive a clonal population. Hybridoma clones that produced anti-human CD52 antibodies were scaled up for
- 10 further development.

Example 2: PCR Analysis of Heavy and Light Chains of Mouse Anti-Human CD52 Antibodies

[0394] A number of mouse anti-human CD52 monoclonal antibodies (FIG. 1B) were identified by testing hybridoma supernatants for the presence of anti-human CD52 reactivity.

- 15 Individual clones were selected and the mouse heavy and light chain variable sequences were identified by PCR cloning and sequencing. The sequences of the light chains are shown in FIG. 2 as compared to YTH 34.5 HL (*i.e.*, Campath IG Kappa (rat) and a reagent antibody CF1D12 (CF1D12 Kappa) (Invitrogen Life Science Technologies). Similarly, the sequences of the heavy chains are shown in FIG. 3 as compared to YTH 34.5 HL and a reagent antibody CF1D12.

- 20 [0395] A total of 10 Zunique light chain variable sequences and 11 unique heavy chain variable sequences were identified. If one includes Campath® and CF1D12, 7 unique CDR-1 regions (Table 1), 8 unique CDR-2 regions (Table 2) and 7 unique CDR-3 regions (Table 3) were identified within the light chains of anti-human CD52 antibodies.

25 Table 1: Light Chain CDR-1 Sequences

Light Chain CDR-1	Sequence
A	KASQNIDKYLN (SEQ ID NO: 27)
B	KSSQSLLESDGRTYLN (SEQ ID NO: 28)
C	KSSQSLLDSDGKTYLN (SEQ ID NO: 29)
D	KSSQSLLDSDGRTYLN (SEQ ID NO: 30)
E	KSSQSLLYSNGKTYLN (SEQ ID NO: 31)
F	RSSQSLVHTNGNSYLH (SEQ ID NO: 32)
G	RSSQSLVHTNGNTYLH (SEQ ID NO: 33)

Table 2: Light Chain CDR-2 Sequences

Light Chain CDR-2	Sequence
A	NTNNLQT (SEQ ID NO: 34)
B	LVSNLDS (SEQ ID NO: 35)
C	LVSKLDS (SEQ ID NO: 36)
D	LVSNLGS (SEQ ID NO: 37)
E	LVSALDS (SEQ ID NO: 38)
F	LVSNLNS (SEQ ID NO: 39)
G	LVSHLDS (SEQ ID NO: 40)
H	MVSNRFS (SEQ ID NO: 41)

Table 3: Light Chain CDR-3 Sequences

Light Chain CDR-3	Sequence
A	LQHISRPRT (SEQ ID NO: 42)
B	WQGTHFPWT (SEQ ID NO: 43)
C	VQGSHFHT (SEQ ID NO: 44)
D	VQGTRFHT (SEQ ID NO: 45)
E	VQGTHLHT (SEQ ID NO: 46)
F	SQSTHVPFT (SEQ ID NO: 47)
G	SQSAHVPPLT (SEQ ID NO: 48)

[0396] If one includes Campath® and CF1D12, a total of 8 unique CDR-1 regions (Table 4), 10 unique CDR-2 regions (Table 5) and 8 unique CDR-3 regions (Table 6) have been identified within the heavy chains of anti-human CD52 antibodies.

Table 4: Heavy Chain CDR-1 Sequences

Heavy Chain CDR-1	Sequence
A	GFTFTDFYMN (SEQ ID NO: 49)
B	GFTFSDAWMD (SEQ ID NO: 50)
C	RFTFSDAWMD (SEQ ID NO: 51)
D	GLTFSDAWMD (SEQ ID NO: 52)
E	GFPFSNYWMN (SEQ ID NO: 53)
F	GFTFNKYWMN (SEQ ID NO: 54)
G	GFTFNTYWMN (SEQ ID NO: 55)
H	GFTFTDYMS (SEQ ID NO: 56)

Table 5: Heavy Chain CDR-2 Sequences

Heavy Chain CDR-2	Sequence
A	FIRDKAKGYTTEYNPSVKG (SEQ ID NO: 57)
B	EIRNKAKNHVAYYAESVKG (SEQ ID NO: 58)

C	EIRNKANNHATYYAESVKG (SEQ ID NO: 59)
D	EIRNKAKNHVKYYAESVKG (SEQ ID NO: 60)
E	EIRNKAKNHATYYAESVKG (SEQ ID NO: 61)
F	EIRKKVNNHATYYAESVKG (SEQ ID NO: 62)
G	QIRLKSNNYATHYAESVKG (SEQ ID NO: 63)
H	QIRLKSDNYATHYAESVKG (SEQ ID NO: 64)
I	FIRNKANGYTTEYNASVKG (SEQ ID NO: 65)
J	FIRNKANGYTTEYSASVKG (SEQ ID NO: 66)

Table 6: Heavy Chain CDR-3 Sequences

Heavy Chain CDR-3	Sequence
A	AREGHTAAPFDY (SEQ ID NO: 67)
B	TTLDS (SEQ ID NO: 68)
C	TSLDY (SEQ ID NO: 69)
D	TGLDY (SEQ ID NO: 70)
E	TPIDY (SEQ ID NO: 71)
F	TPVDF (SEQ ID NO: 72)
G	TRYIFFDY (SEQ ID NO: 73)
H	TRYIWFDY (SEQ ID NO: 74)

- 5 [0397] The association of specific light and heavy chain CDR regions within 13 different anti-human CD52 antibodies is depicted in Table 7.

Table 7: Classification of Anti-Human CD52 Antibodies on the Basis of CDR Composition

10

Clone Name	Heavy Chain CDR-1			Light Chain		
	CDR-1	CDR-2	CDR-3	CDR-1	CDR-2	CDR-3
Campath	A	A	A	A	A	A
CF1D12	B	B	B	B	B	B
8G3.25.3.5	C	C	C	C	C	B
GMA 4G7.F3	B	D	C	C	D	B
GMA 9D9.A2	B	E	B	C	E	B
GMA 11C11.C5	B	E	C	C	C	B
GMA 3G7.E9	B	F	C	C	F	B
5F7.1.1.4	D	E	D	D	G	B
12G6.15.1.2	E	G	E	E	C	C
23E6.2.2.1	F	H	E	E	C	D
2C3.3.8.1	G	G	F	E	C	E
7F11.1.9.7	H	I	G	F	H	F
4B10.1.2.4	H	J	H	G	H	G

Clones 8G3.25.3.5, 4G7.F3, 9D9.A2, 11C11.C5, 3G7.E9, 5F7.1.1.4, 12G6.15.1.2, 23E6.2.2.1, 2C3.3.8.1, 7F11.1.9.7 and 4B10.1.2.4 are hereafter referred to as 8G3, 4G7, 9D9, 11C11, 3G7, 5F7, 12G6, 23E6, 2C3, 7F11 and 4B10, respectively.

Table 7.1: SEQ ID NOs of the CDRs of the Anti-Human CD52 Antibodies

Clone Name	Heavy Chain			Light Chain		
	CDR-1	CDR-2	CDR-3	CDR-1	CDR-2	CDR-3
Campath	49	57	67	27	34	42
CF1D12	50	58	68	28	35	43
8G3	51	59	69	29	36	43
4G7	50	60	69	29	37	43
9D9	50	61	68	29	38	43
11C11	50	61	69	29	36	43
3G7	50	62	69	29	39	43
5F7	52	61	70	30	40	43
12G6	53	63	71	31	36	44
23E6	54	64	71	31	36	45
2C3	55	63	72	31	36	46
7F11	56	65	73	32	41	47
4B10	56	66	74	33	41	48

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Example 3: Cloning of Mouse IgG Variable Region Genes from Mouse Hybridoma Cells to Generate a Mouse/Human Chimeric IgG1 Antibody

[0398] Actively proliferating and antibody secreting hybridoma cells were used to isolate RNA using Trizol reagent (Gibco/BRL) following the manufacturer's suggested protocol. RNA was quantified by measuring OD using Nanodrop, and the integrity of the RNA was determined by running it on a gel or by using a bioanalyzer. Total RNA was reverse transcribed to cDNA and the variable regions for the heavy and light chains were amplified by polymerase chain reaction (PCR). The cDNA was generated using BD Sprint PowerScript Reverse Transcriptase (Clontech) and Oligo(dT) primer at 0.5 µg/µl (Invitrogen Cat# Y01212) and reverse primers (located in the constant region of the heavy and light chains) listed numerically below at 10 µM following the manufacturer's protocol. Specifically, primers numbered 3 (SEQ ID NO: 77), 11 (SEQ ID NO: 85), 19 (SEQ ID NO: 93), 20 (SEQ ID NO: 94) and 21 (SEQ ID NO: 95) were employed. PCR amplification of the heavy and light chain variable regions was carried out using cDNA generated as described above. 1 µl of cDNA was mixed with forward primer and reverse primers at 10 µM each for both heavy and light chains and mixed with PCR super mix (Invitrogen) in the presence of 2 µl of MgCl₂ at 25 mM. The PCR program was run in the following steps: 1) 95°C for 2 minutes; 2) 95°C for 30 seconds; 3) 56°C for 30 seconds; 4) 68°C for 45 seconds; 5) Repeat steps 2 to 4 25 times; 6) 68°C for 10 minutes and hold at 16°C. The PCR product was analyzed on a 2% gel for the presence of variable region sequence product of

about 300-400 bp in size and the appropriate bands were cloned into pCR2.1-TOPO TA cloning Kit (Invitrogen) following the manufacturer's instructions and the cloned and sequence confirmed using M13 primers. Primers used for reverse transcribing and for PCR amplification of light chain and heavy chain sequences are provided:

- 5 Light chain primers
- [0399] 1) Lead-ML kappa = 5' ATGGGCWTC AARATGRARWCWCAT 3' (Forward primer in leader sequence) (SEQ ID NO: 75)
- [0400] 2) FR1-ML kappa = 5' GAYATTGTGMTRACMCARKMTCAA 3' (Forward primer in the frame work 1) (SEQ ID NO: 76)
- 10 [0401] 3) ML kappa const = 5' ACTGGATGGTGGGAAGATGGA 3' (Reverse primer in constant region) (SEQ ID NO: 77)
- [0402] 4) VK-MK = 5' GAYATTGTGMTSACMCARWCTMCA 3' (Forward primer in the frame work 1) (SEQ ID NO: 78)
- [0403] 5) MKC-Const = 5' GGATACAGTTGGTGCAGCATC 3' (Reverse primer in constant region) (SEQ ID NO: 79)
- 15 Heavy chain primers
- [0404] 6) MH-SP-ALT1 = 5' ATGRASTTSKGGYTMARCTKGRTT 3' (Forward primer in leader sequence) (SEQ ID NO: 80)
- [0405] 7) MH-SP-ALT2 = 5' ATGRAATGSASCTGGGTYWTYCTCT 3' (Forward primer in leader sequence) (SEQ ID NO: 81)
- 20 [0406] 8) MH-FR1 = 5' SAGGTSMARCTGCAGSAGTCT 3' (Forward primer in the frame work 1) (SEQ ID NO: 82)
- [0407] 9) MH-FR1-1 = 5' SAGGTGMAGCTCSWRSARYCSGGG 3' (Forward primer in the frame work 1) (SEQ ID NO: 83)
- 25 [0408] 10) MH-J2 = 5' TGAGGAGACTGTGAGAGTGGTGCC 3' (Reverse primer in J region) (SEQ ID NO: 84)
- [0409] 11) MH-gamma-const = 5' AYCTCCACACACAGGRRCCAGTGGATAGAC 3' (Reverse primer in constant region) (SEQ ID NO: 85)
- [0410] 12) VH MH1 = 5' SARGTNMAGCTGSAGSAGTC 3' (Forward primer in the frame work 1) (SEQ ID NO: 86)
- 30

- [0411] 13) VH MH2 = 5' SARGTNMAGCTGSAGSAGTCWGG 3' (Forward primer in the frame work 1) (SEQ ID NO: 87)
- [0412] 14) VH MH3 = 5' CAGGTTACTCTGAAAGWGTSTG 3' (Forward primer in the frame work 1) (SEQ ID NO: 88)
- 5 [0413] 15) VH MH4 = 5' GAGGTCCARCTGCAACARTC 3' (Forward primer in the frame work 1) (SEQ ID NO: 89)
- [0414] 16) VH MH5 = 5' CAGGTCCAACVCAGCARCC 3' (Forward primer in the frame work 1) (SEQ ID NO: 90)
- [0415] 17) VH MH6 = 5' GAGGTGAASSTGGTGAATC 3' (Forward primer in the frame work 1) (SEQ ID NO: 91)
- 10 [0416] 18) VH MH7 = 5' GATGTGAACTTGAAGTGTC 3' (Forward primer in the frame work 1) (SEQ ID NO: 92)
- [0417] 19) IgG1 = 5' ATAGACAGATGGGGGTGTCGTTTTGGC 3' (Reverse primer in mouse IgG1 CH1 constant region) (SEQ ID NO: 93)
- 15 [0418] 20) IgG2A = 5' CTTGACCAGGCATCCTAGAGTCA 3' (Reverse primer in mouse IgG2A CH1 constant region) (SEQ ID NO: 94)
- [0419] 21) IgG2B = 5' AGGGGCCAGTGGATAGAGTGATGG 3' (Reverse primer in mouse IgG2B CH1 constant region) (SEQ ID NO: 95)
- [0420] Degenerate primers led to some degeneracy in the 5' end of the frame work 1 region of both heavy and light chains. The consensus DNA sequence from several independent heavy chain variable region clones and from light chain variable region clones was used to derive the amino acid sequence.
- 20 [0421] Functional chimeric anti-CD52 antibodies were produced by joining the heavy chain and light chain variable regions to the DNA encoding human IgG1 heavy chain (identical sequence to that found in Campath-1H[®]) and human kappa light chain constant region (identical sequence to that found in Campath-1H[®]), respectively. To generate pCEP4 (Invitrogen) light chain vector encoding CD52 antibody light chain, the light chain variable sequence was PCR amplified and engineered by ligase independent cloning into the pCEP4 LIC light chain vector to have the human kappa signal sequence in the 5' end and the light chain constant region in the 3' end. Similarly, to generate pCEP4 heavy chain vector, the variable region of the heavy chain sequence was engineered by ligase independent cloning into the pCEP4 LIC heavy chain vector to have the human kappa chain signal sequence in the 5' end and the heavy chain constant
- 30

region encompassing CH1, hinge, CH2 and CH3 regions in the 3' end. The constant region amino acid sequences for both heavy and light chains are identical to that of the constant regions present in Campath1H antibody.

[0422] Briefly, pCEP4 LIC vector was digested with BfuA1 (New England Biolabs-NEB) in appropriate buffer following the manufacturer's recommendations and after complete digestion, the vector was purified using PureLink PCR Purification Kit (Invitrogen). The linearized plasmid was then treated with T4 DNA polymerase (New England Biolabs) to generate single-stranded ends and was used to clone the variable region fragment. Heavy chain specific pCEP4 LIC vector was used for cloning heavy chain variable region and light chain specific pCEP4 LIC vector was used for cloning light chain variable region. Variable region insert was generated by PCR using pCR2.1-TOPO heavy chain variable region containing plasmid or pCR2.1-TOPO light chain variable region containing plasmid as template and primers that contain variable chain specific sequence and vector overhangs. VENT DNA polymerase (New England Biolabs) was used for PCR amplification of the insert. PCR-amplified insert was gel purified and treated with T4 DNA polymerase to generate single-stranded ends. Prepared vectors for heavy chain and light chain and respective variable region insert fragments were combined and incubated at room temperature for 10 minutes and used to transform TOPO10 cells (Invitrogen), ampicillin resistant colonies were picked and sequence verified. pCEP4 heavy chain and pCEP4 light chain clones that had the correct heavy chain and light chain sequences inserted in-frame were amplified and used for protein production. The heavy chain construct was co-transfected with the corresponding light chain construct in a 1:1 ratio into HEK293 cells (Invitrogen) using the cationic lipid LipofectamineTM 2000 (Invitrogen). The conditioned medium was harvested three days after the transfection and the chimeric antibody was purified using protein A chromatography. For this chromatography method, the medium was added to protein A and washed with 50 column volumes of PBS. The chimeric antibody was eluted with 5 column volumes of 12.5 mM citric acid, pH 3.0. The pH of the eluted antibody was neutralized by addition of 0.5 M HEPES. The buffer was exchanged into PBS by using a PD-10 gel filtration column.

Example 4: Analysis of the Epitope Specificities of Chimeric anti-Human CD52

30 Monoclonal Antibodies

[0423] The epitope specificities of the clones were determined by assessing the ability of the chimeric antibodies to bind to a panel of cell lines engineered to express mutants of human

CD52 (FIG. 4) generated by alanine scanning mutagenesis. Antibody substitution of the first 10 amino acids of the 12 amino acid extracellular region of CD52 was conducted on human CD52 cDNA in pcDNA3.1 expression vector (Invitrogen) using the STRATAGENE QUIKCHANGE II XL site-directed mutagenesis kit. pcDNA3.1 vector encoding wild type or mutant CD52

5 sequence was sequence-verified and transfected into CHO cells using LipofectamineTM and by selecting in media containing G418 to generate CHO cell lines that expressed wild type or alanine mutant CD52. Epitope specific binding of anti-human CD52 chimeric antibodies was determined by measuring the binding of the antibodies against the wild type and mutant CD52 expressing cells by FACS. FACS analysis was carried out by detecting the binding of chimeric

10 anti-CD52 antibodies using PE-conjugated goat anti-human secondary antibody (Jackson ImmunoResearch Labs). FIGS. 5A-5C show the Mean Fluorescence Intensity (MFI) of anti-CD52 monoclonal antibodies to wild type and mutant CD52 expressing cell lines. Even though CD52 is a very short, 12 amino acid, GPI anchored protein, the FACS results clearly define that there are three sets of antibodies: (1) N-terminal binding group (such as 4B10); (2) middle

15 binding group (such as 4G7, 9D9 and 11C1) and (3) C-terminal binding group (such as 23E6, 12G6, and 2C3). The epitope specificities of the anti-human CD52 monoclonal antibodies (identified by the abbreviated names described at the end of Example 2) are summarized in Table 8.

Table 8: Characteristics of 11 Mouse Anti-Human CD52 Monoclonal Antibodies

Clone	Isotype	Epitope Specificity
Rat YTH34.5HL	IgG2a	9-10-11-12
Mouse CF1D12	IgG3	3-4-5-6-7
8G3.25.3.5	IgG3	Not confirmed
4G7.F3	IgG3	3-4-5-6-7
9D9.A2	IgG3	3-4-5-6-7
11C11.C5	IgG3	1-3-4-5-6-7
3G7.E9	IgG2b	1-3-4-5-6-7
5F7.1.1.4	IgG3	1-3-4-5-6-7-10
12G6.15.1.2	IgG3	7-8-9
23E6.2.2.1	IgG3	7-8-9
2C3.3.8.1	IgG3	7-8-9-10
7F11.1.9.7	IgG1	1-2-3-4-5
4B10.1.2.4	IgG2a	1-2-3-4-5

- [0424] CD52 is an extremely small antigen but possesses a relatively large, hydrophilic N-linked glycan moiety as well as a hydrophobic GPI-anchor. To explore the possibility that the sugars might constitute all or part of an epitope recognized by the anti-CD52 antibodies, samples of affinity purified CD52 from CHO-CD52 cells were treated with the endoglycosidase,
- 5 PNGase-F, to completely remove N-linked sugars from the antigen. Treated and mock-treated control samples were then resolved by SDS-PAGE, blotted to polyvinylidene fluoride (PVDF) membrane (Invitrogen), probed with 3µg/ml final of each of the anti-CD52 chimeric monoclonal antibodies indicated, and subsequently developed according to standard western blotting procedures using enhanced chemiluminescent detection. Blots with Campath-1H® (C1H) and
- 10 with secondary antibody alone (2° Alone) were run as positive and negative controls, respectively, and probed with each of the monoclonal antibodies (FIG. 5D). The results revealed different binding preferences amongst the antibodies for glycosylated versus de-glycosylated CD52. This characterization allowed for the categorization of the eleven antibodies into four types of binding groups:
- 15 1. Antibodies exhibiting binding with no apparent preference for glycosylated versus de-glycosylated CD52 (4G7, 9D9)
 2. Antibodies exhibiting binding specific for glycosylated CD52 (7F11, 4B10)
 3. Antibodies exhibiting binding specific for de-glycosylated CD52 (8G3)
 4. Antibodies exhibiting binding preferential for de-glycosylated over glycosylated CD52
 - 20 (12G6, 5F7, 23E6, 2C3, 11C11, 3G7)

Example 5: CDC Activity of Chimeric Anti-CD52 Antibodies

- [0425] A complement-dependent cytotoxicity (CDC) assay was performed as described µbelow. Briefly, CHO K1 cells engineered to express CD52 protein (CHO-CD52) were used as
- 25 target cells and labeled with Na₂⁵¹CrO₄ (New England Nuclear, Boston, MA) at 37°C for 1-2 hrs. The cells were washed, resuspended with X-Vivo media, and mixed with anti-human CD52 antibodies to final concentration of 2.2 µg/ml. Human complement (Sigma) was added to the experimental wells to a final concentration of 10%. After a 1-5-hour incubation, 25 µl of cell-free supernatant was collected from each well and counted in a MICROBETA TRILUX
- 30 Scintillation Counter (Wallac, Gaithersburg, MD). The amount of ⁵¹Cr spontaneously released was obtained by incubating target cells in medium alone. Spontaneous release from target cells

was typically less than 20%. The total amount of ^{51}Cr incorporated was determined by adding 1% Triton X-100 in distilled water, and the percentage lysis was calculated as follows: [(sample counts per minute (c.p.m.) -spontaneous c.p.m.)/(total c.p.m.-spontaneous c.p.m.)] X 100.

- [0426] Twelve different chimeric anti-CD52 antibodies (mouse variable region and human IgG1 constant region) were tested in CDC assay with human complement on CHO-CD52 cells. Campath-1H® humanized antibody was used as a positive control. A negative control was Campath-1H® null (a non-cell-binding minimal mutant of Campath-1H® – two point mutations in H2 loop-heavy chain CDR2 region (K52bD and K53D; Gilliland LK *et al.*, *Journal of Immunology*, 162:3663-3671 (1999)). The results indicate that the chimeric antibodies are capable of mediating CDC killing on CD52-expressing cells. Some of the chimeric antibodies mediated robust killing equivalent or better than Campath® (FIG. 6).

Example 6: ADCC Activity of Chimeric Anti-CD52 Antibodies

- [0427] An antibody-dependent cytotoxicity (ADCC) assay was performed as described below. Briefly, CHO K1 cells engineered to express CD52 protein (CHO-CD52) were used as target cells and labeled with $\text{Na}_2^{51}\text{CrO}_4$ (New England Nuclear, Boston, MA) at 37°C for 1-2 hrs. The cells were washed, resuspended with X-Vivo media, and mixed with anti-human CD52 antibodies to final concentration of 1.1 µg/ml. Human PBMC were used as effector cells and were added at a 1:100 target-to-effector cell ratio. After a 6 hr-overnight incubation, 25 µl of cell-free supernatant was collected from each well and counted in a MICROBETA TRILUX Scintillation Counter (Wallac, Gaithersburg, MD). The amount of ^{51}Cr spontaneously released was obtained by incubating target cells in medium alone. Spontaneous release from target cells was typically less than 20%. The total amount of ^{51}Cr incorporated was determined by adding 1% Triton X-100 in distilled water, and the percentage lysis was calculated as follows: [(sample c.p.m.-spontaneous c.p.m.)/(total c.p.m.-spontaneous c.p.m.)] X 100.

- [0428] Twelve different chimeric anti-CD52 antibodies (mouse variable region and human IgG1 constant region) were tested in ADCC assay using human PBMC as effector cells. Campath-1H® humanized antibody was used as a positive control. Used as a negative control was Campath-1H® null (a non-cell-binding minimal mutant of Campath-1H® – two point mutations in H2 loop-heavy chain CDR2 region (K52bD and K53D; Gilliland, 1999, *supra*). The results indicate that the chimeric antibodies are capable of mediating ADCC killing on CD52-expressing cells. Some of the chimeric antibodies mediated robust killing equivalent or better than Campath-1H® (FIG. 7).

Example 7: Evaluation of the Binding of Chimeric Anti-CD52 Antibodies to Defined Lymphocyte Population

- [0429] The following fluorochrome conjugated antibodies were used for flow cytometric analysis: anti-CD3-FITC, anti-CD27-PE, anti-CD62L-PE Cy5, anti-CD56- PE Cy7, anti-CD16-
 5 APC Cy7 (BD Biosciences, San Diego, CA), anti-CD45RA-ECD (Beckman Coulter), anti-CD19-Pacific Blue, anti-CD4-APC Cy5.5 and anti-CD8 pacific orange (Invitrogen, CA). All the mouse chimeric anti-human CD52 antibodies as well as the humanized Campath-1H® were conjugated to Alexa fluor 647 (BD Pharmingen). Healthy human peripheral blood mononuclear cells were obtained either from cryopreserved buffy coats or from mononuclear cells separated
 10 from blood of normal donors obtained from commercial vendors (Bioreclamation, NY, USA). For enrichment of mononuclear cells, human peripheral blood was diluted 1:1 with sterile phosphate buffered saline (PBS) and carefully layered over Ficoll-hypaque (GE Healthcare Bio-Sciences, Uppsala, Sweden) and centrifuged for 30 min at room temperature. The interphase layer of mononuclear cells was drawn out and washed in PBS containing 5% fetal bovine serum
 15 (FACS buffer). Contaminating red blood cells (RBCs) were lysed with RBC lysing solution (Sigma, St. Louis, MO, USA). Cells were resuspended in cold FACS buffer and the debris was removed using a 40 micron filter. Ten color flow cytometry was performed to evaluate the binding ability of 9 chimeric anti-human CD52 antibodies (4B10, 7F11, 9D9, 5F7, 2C3, 4G7, 23E6, 8G3, 3G7) as compared to Campath-1H®.
- 20 [0430] Briefly, replicates of 1×10^6 PBMC's in FACS buffer were incubated with a cocktail of pre-titrated dilutions of antibodies against CD3, CD27, CD45RA, CD62L, CD56, CD19, CD8, CD4, CD16 along with one of 9 chimeric anti-human CD52 antibodies (4B10, 7F11, 9D9, 5F7, 2C3, 4G7, 23E6, 8G3, 3G7) for 30 min at 4°C. Cells were washed and fixed in PBS containing 1% paraformaldehyde. 100,000 events of the stained cells were acquired on BD LSR-II (BD
 25 Biosciences, San Jose, CA) and the data was analyzed using FlowJo 7.2 version Software (Tree Star, Inc, Oregon, USA). Multiple subsets with distinct phenotypic characteristics have been defined among B and T lymphocytes and CD52 has been shown to be expressed on all human lymphocytes. Ten color flow cytometry analysis was performed to identify the lymphocyte subsets, and to assess similarities and the differences in the binding characteristics of anti-CD52
 30 antibodies to cell surface CD52 on defined subsets. Using a combination of markers, 11 phenotypically distinct cell populations corresponding to B, T and NK cell lineages were first defined from the lymphocyte gate. The intensity of staining which corresponds to the ability of

anti-CD52 antibodies to detect CD52 expression was then assessed. The histograms (FIGS. 8A-8C) show a comparison of the level of detection of CD52 with each antibody on individual lymphocyte populations. The data shows that the antibodies exhibit significant differences in binding to CD52. The level of detection with 4B10, 9D9, 7F11 and Campath-1H® are comparable, although 4B10 consistently shows the highest level of detection than other antibodies including Campath-1H®, on almost all the cell subsets examined. On the other hand, the detection level of CD52 with 3G7, 4G7, 8G3 and 23E6 antibodies is significantly lower. The results indicate a hierarchy within the antibodies with respect to their ability to recognize CD52 on different cell populations with 4B10 being highest and 3G7 being the lowest. Interestingly, these differences are less obvious on CD4 effector and more so on NK cell subsets on which CD52 appears to be expressed at relatively lower levels. The variations in the binding characteristics indicate that the properties of the chimeric antibodies not only differ significantly from Campath-1H® but also reflect differences in properties among the antibodies.

Example 8: Analysis of Chimeric Anti-CD52 Antibodies in Human CD52 Transgenic Mice (7F11, 8G3, 23E6, 12G6, 4B10 and 5F7)

[0431] Human CD52 transgenic mice were administered either Campath® or chimeric anti-CD52 antibodies (7F11, 8G3, 23E6, 12G6, 4B10 and 5F7) to examine the level of lymphocyte depletion. Mice were injected intra-peritoneally with either Campath® or the chimeric anti-CD52 antibodies in a 100 µl volume at a dose of 1 mg/kg. Three days later mice were sacrificed and blood and spleens were collected to determine the level of B and T-cell depletion. Flow cytometry was utilized to evaluate the absolute numbers of total T helper cells, cytotoxic T cells, and B cells present in the circulating peripheral blood or spleens of huCD52 transgenic mice. These lymphocyte populations were defined by their surface expression of the following protein antigens: CD4 expression identifies the T helper cell population, CD8 expression identifies the cytotoxic T cell population and CD19 expression identifies all mature B cell populations. A significant level of T and B-cell depletion was observed for both the 12G6 and 4B10 antibodies, which was comparable to the depletion observed with Campath®. Treatment with either Campath®, the chimeric 12G6 or the chimeric 4B10 antibody significantly reduced T and B cells in both the blood and spleens of treated mice at this dose level. The 7F11 and 5F7 chimeric antibodies resulted in significant levels of T cell depletion level in the blood and spleen but were less effective at depleting B cells in both compartments. Treatment with the 23E6 antibody

resulted in a moderate level of depletion at this dose while little to no depletion was observed with the lower affinity 8G3 antibody.

[0432] Figs. 9A-9C show the level of CD4 T cells, CD8 T cells and CD19 B cells in the blood 72 hours after dosing with the chimeric antibodies. Figs. 10A-10C show the level of CD4 T cells, CD8 T cells and CD19 B cells in the spleen 72 hours after dosing.

Example 9: Analysis of Chimeric Anti-CD52 Antibodies in Human CD52 Transgenic Mice (2C3, 3G7, 4B10, 9D9, and 11C11)

[0433] Human CD52 transgenic mice were administered either Campath® or chimeric anti-CD52 antibodies (2C3, 3G7, 4B10, 9D9 and 11C11) to examine the level of lymphocyte depletion. Mice were injected intravenously with either Campath® or the chimeric anti-CD52 antibodies in a 100 µl volume at a dose of 1 mg/kg. Three days later mice were sacrificed and blood and spleens were collected to determine the level of B and T-cell depletion. Flow cytometry was utilized to evaluate the absolute numbers of total T helper cells, cytotoxic T cells, and B cells present in the circulating peripheral blood of huCD52 transgenic mice. These lymphocyte populations were defined by their surface expression of the following protein antigens: CD4 expression identifies the T helper cell population, CD8 expression identifies the cytotoxic T cell population and CD19 expression identifies all mature B cell populations. A significant level of T and B cell depletion was observed for several antibodies in both the blood and spleen. The depleting activity for 2C3 and 9D9 was comparable to that observed with Campath® with significant levels of CD4 and CD8 T cells and CD19 B cells being depleted. Treatment with chimeric 4B10 also resulted in a significant decrease in the numbers of lymphocytes in the blood of transgenic mice. While treatment with either the chimeric antibody 3G7 or 11C11 antibody significantly depleted T cells in the blood, the level of B cells present were not significantly affected at this dose.

[0434] FIGS. 11A-11C show the level of CD4 T cells, CD8 T cells and CD19 B cells in the blood 72 hours after dosing with the chimeric antibodies.

Example 10: Analysis of the Efficacy of Anti-CD52 Antibodies (7F11, 4B10 and 12G6)

[0435] Forty SCID mice (n=8 per group) were injected with 1×10^6 B104 tumor cells in 100 µl volume on the right flank. On day 11 post tumor cell injection, treatment began with Campath®, 7F11, 4B10 or 12G6 chimeric antibodies. Antibodies were administered once weekly at 10 mg/kg by intraperitoneal injection throughout the remainder of the experiment. All mice in the untreated group developed progressively growing tumors requiring sacrifice with a median

survival of 29 days. Treatment with Campath® resulted in a statistically significant increase in survival compared to the untreated group (median survival (MS) of 50 days and $p < 0.0001$). Treatment with the chimeric anti-CD52 antibodies also resulted in a statistically significant increase in survival compared to untreated mice ($p < 0.0001$ for 7F11 and 4B10 and $p = 0.0020$ for 12G6). Based on survival rates, the activity of both 7F11 and 4B10 antibodies appears to be greater than Campath® (63% survival for 7F11 and 75% survival for 4B10 compared to 50% survival for Campath®). FIG. 12 shows the percent survival of the mice after treatment.

Example 11: Analysis of the Efficacy of Anti-CD52 Antibodies (2C3, 8G3 and 23E6)

[0436] Forty SCID mice ($n=8$ per group) were injected with 1×10^6 B104 tumor cells in a 100 μ l volume on the right flank. On day 11 post tumor cell injection, treatment began with either Campath®, 2C3, 8G3 or 23E6 chimeric antibodies. Antibodies were administered once weekly at 10 mg/kg by intraperitoneal injection throughout the remainder of the experiment. All mice in the untreated group developed progressively growing tumors requiring sacrifice with a median survival of 26 days. Treatment with Campath®, 23E6, and the 2C3 antibody resulted in statistically significant increases in survival ($p=0.0025$, $p=0.0007$, and $p=0.0002$ respectively). FIG. 13 shows the percent survival of the mice after treatment.

Example 12: Analysis of the Efficacy of Chimeric Anti-CD52 Antibodies in a Xenograft Tumor Model (9D9 and 4B10)

[0437] Forty SCID mice ($n=8$ per group) were injected with 1×10^6 B104 tumor cells in a 100 μ l volume on the right flank. On day 11 post tumor cell injection, treatment began with either Campath®, 9D9 or 4B10 chimeric antibody. Antibodies were administered once weekly at 10 mg/kg by intraperitoneal injection throughout the remainder of the experiment. All mice in the untreated group developed progressively growing tumors requiring sacrifice with a median survival of 27 days. Treatment with Campath® resulted in a statistically significant increase in survival compared to the untreated group (median survival not achieved and $p < 0.0001$). Treatment with the chimeric anti-CD52 antibodies also resulted in a statistically significant increase in survival compared to untreated mice ($p < 0.0001$ for 9D9 and 4B10). Statistical analysis of the survival curves reveals that the 9D9 chimeric antibody displayed activity comparable to Campath® ($p=0.0675$) in this experiment. FIG. 14 shows the percent survival of the mice after treatment.

Example 13: Analysis of the Efficacy of Chimeric Anti-CD52 Antibodies in a Xenograft Tumor Model (2C3 and 11C11)

[0438] Forty SCID mice (n=8 per group) were injected with 1×10^6 B104 tumor cells in a 100 μ l volume on the right flank. On day 11 post tumor cell injection, treatment began with either Campath[®], 2C3 or 11C11 chimeric antibody. Antibodies were administered once weekly at 10 mg/kg by intraperitoneal injection throughout the remainder of the experiment. All mice in the untreated group developed progressively growing tumors requiring sacrifice with a median survival of 32 days. Treatment with Campath[®] resulted in a statistically significant increase in survival compared to the untreated group (median survival not achieved and $p < 0.0001$).

10 Treatment with the chimeric anti-CD52 antibodies also resulted in a statistically significant increase in survival compared to untreated mice ($p < 0.0001$ for 2C3 and $p = 0.0004$ for 11C11). Statistical analysis of the survival curves reveals that both the 2C3 and 11C11 chimeric antibodies displayed activity comparable to Campath[®] ($p = 0.3173$ for 2C3 and $p = 0.9703$ for 11C11). FIG. 15 shows the percent survival of the mice after treatment with Campath[®], 2C3

15 chimeric antibody or 11C11 chimeric antibody.

Example 14: Generation and Analysis of Humanized Anti-CD52 Antibody 4B10

[0439] Humanized anti-human CD52 antibody 4B10 was generated by grafting the CDR regions from the mouse 4B10 antibody into a human antibody variable region framework. Mouse 4B10 heavy chain and light chain sequences were evaluated by a web-based sequence alignment in order to identify a human germline heavy chain and light chain framework sequence that would serve as a suitable acceptor for the CDR graft (FIG. 16). The residues defining the CDR regions by Kabat and IMGT[®] were superimposed into human framework regions that have high sequence identity to generate humanized heavy chain and light chain sequences. Visual inspection and sequence analysis of the superimposed 4B10 heavy and light

20 chain sequences was carried out to identify the most suitable acceptor sequence. Of all the germline sequences that have high similarity, the VH3-72 germline sequence for heavy chain and the VK2-A18b for light chain (human germ line sequences can be found at the website described in the publication by Tomlinson, IM, *et al.*, *EMBO J.*, 14(18):4628-4638 (1995); Cook, GP., *et al.*, *Nature Genetics*, 7:162-168 (1994)) were selected from their high degree of

25 homology, sequence similarity to mouse framework regions and for minimal disruption of CDR loop structure as CDR acceptor sequence. CDR1, 2, and 3 sequences of heavy chain and light chain for 4B10 were grafted into VH3-72 and VK2-A18b human framework regions

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respectively to generate humanized heavy chain and light chain sequences for 4B10 (illustrated in FIG. 17; FIG. 110).

Example 15: Assessment of the Binding Activities of Chimeric and Humanized 4B10 Monoclonal Antibodies

5 [0440] Chimeric and humanized 4B10 antibodies were produced and purified as described in Example 3 and analyzed for their ability to bind to the B cell line B104, which endogenously expresses CD52, by FACS. Briefly, 2×10^5 B104 cells were incubated with antibody (0.02 $\mu\text{g/ml}$ to 16.7 $\mu\text{g/ml}$) in PBS containing 5% fetal bovine serum and 5% goat serum. The bound antibody was detected with FITC labeled goat anti-human secondary antibody which detected
10 chimeric or humanized anti-CD52 antibodies. Labeled cells were analyzed using a FACSCalibur system (Becton Dickinson). FIG. 18 shows the fold increase in Geometric mean fluorescence intensity of each sample normalized (divided) to that of 2°-only sample. The 11 different concentrations (12th point on X axis is secondary alone) of the humanized and chimeric antibody used in the assay is shown on the X axis and the Geo Mean fold increase in the mean
15 fluorescence is on the Y axis. The results indicate that the humanized 4B10 antibody bound as well or slightly better than chimeric 4B10 antibody to CD52 expressing cells.

Example 16: Assessment of the ADCC Activities of Chimeric and Humanized 4B10 Monoclonal Antibodies

[0441] Humanized and chimeric 4B10 antibodies were evaluated for their ability to mediate
20 ADCC killing of CD52-expressing cells. An ADCC assay was carried out as described above in Example 6. Briefly, CHO K1 cells engineered to express CD52 protein (CHO-CD52) were used as target cells and labeled with $\text{Na}_2^{51}\text{CrO}_4$ (New England Nuclear, Boston, MA) at 37°C for 1-2 hrs. The cells were washed, resuspended in RPMI 1640 media with 10% FCS, and mixed with chimeric or humanized 4B10 antibodies at various concentrations ranging from 10 $\mu\text{g/ml}$ to 0.01
25 $\mu\text{g/ml}$. Human PBMC were used as effectors cells and were added at 1:50 target-to-effector cell ratio. After a 6hr-overnight incubation, 25 μl of cell-free supernatant was collected from each well and counted in a MICROBETA TRILUX Scintillation Counter (Wallac, Gaithersburg, MD). The amount of ^{51}Cr spontaneously released was obtained by incubating target cells in medium alone. Spontaneous release from target cells was typically less than 20%. The total
30 amount of ^{51}Cr incorporated was determined by adding 1% Triton X-100 in distilled water, and the percentage lysis was calculated as follows: $[(\text{sample c.p.m.} - \text{spontaneous c.p.m.}) / (\text{total c.p.m.} - \text{spontaneous c.p.m.})] \times 100$.

[0442] FIG. 19 illustrates the concentrations of control, chimeric and humanized 4B10 antibodies used in the assay (X axis) and the Y axis shows % specific killing. The results indicate that humanized 4B10 antibody mediated equivalent or slightly better ADCC killing than chimeric 4B10 antibody. The control IgG1 isotype control showed only low levels of background killing at the concentrations tested.

Example 17: Assessment of the CDC Activities of Chimeric and Humanized 4B10 Monoclonal Antibodies

[0443] Humanized and chimeric 4B10 antibodies were evaluated for their ability to mediate cytotoxic effect on B104 cells that endogenously express CD52 in the presence of human complement. CellTiter Glo kit (Promega) was used to determine the live cells remaining in the assay. Briefly, B104 cells (target cells) were plated at 2.5×10^4 cells/well in a 96 well plate and were mixed with chimeric or humanized 4B10 antibody at various concentrations ranging from 1 μ g/ml to 25 μ g/ml and human complement to a final concentration of 10%. Complement alone without the antibody and antibody alone without complement were used as controls to determine the background. After three hours of incubation at 37°C, plates were centrifuged for 3 min at 1500 rpm and the live cells present in the pellet were determined using CellTiter Glo assay. Plates were read on Envision machine. FIG. 20 shows the live cells present in the assay as measured using CellTiter Glo assay. Again, with the increasing concentrations of the humanized and chimeric 4B10 antibody there is a decrease in the number of live cells. These results suggest that the humanized antibody performed as well as or slightly better than chimeric 4B10 antibody in CDC mediated killing of B104 cells.

Example 18: Analysis of Pharmacokinetic Profile of Chimeric And Humanized Anti-CD52 Antibodies in CD52 Transgenic Mice (12G6, 7F11, Chimeric And Humanized 4B10)

[0444] Human CD52 transgenic mice were administered one of Campath®, 12G6, 7F11, and chimeric and humanized 4B10 anti-CD52 antibodies to examine the level of lymphocyte depletion. Mice were injected intravenously with one of those antibodies in a 100 μ l volume at a dose of 1 mg/kg. For analysis of anti-antibody responses, 100 μ l of blood was collected into serum separator tubes via puncture of the retro-orbital plexus at 2 hours, 1, 2, 4, 7, and 10 days post antibody injection. ELISA analysis was used to determine the level of circulating human IgG1 in each serum sample. Based on circulating levels of antibody, there appears to be little to no difference between Campath®, 7F11, and the chimeric and humanized forms of 4B10. The 12G6 antibody displayed lower c_{max} values following injection, suggesting that this antibody

may be degraded more quickly. FIG. 21 shows the pharmacokinetic profile of Campath®, 12G6 (chimeric), 7F11 (chimeric), 4B10 (chimeric) and 4B10 (humanized) antibodies.

Example 19: Analysis of the Depleting Activity of Chimeric and Humanized Anti-CD52 Antibodies in CD52 Transgenic Mice (Chimeric and Humanized 4B10)

5 [0445] Human CD52 transgenic mice were administered either Campath® or chimeric or humanized 4B10 anti-human CD52 antibody to examine the level of lymphocyte depletion. Mice were injected intravenously with either Campath® or the chimeric or humanized 4B10 anti-human CD52 antibody in a 100 µl volume at a dose of 0.1 mg/kg. Three days later mice were sacrificed and blood and spleens were collected to determine the level of B and T-cell
10 depletion. Flow cytometry was utilized to evaluate the absolute numbers of total T helper cells, cytotoxic T cells, and B cells present in the circulating peripheral blood of the huCD52 transgenic mice. These lymphocyte populations were defined by their surface expression of the following protein antigens: CD4 expression identifies the T helper cell population, CD8 expression identifies the cytotoxic T cell population and CD19 expression identifies all mature B
15 cell populations. Comparison of the depleting activity in the spleen revealed that there was no difference in the level of T cells depleted following administration of either Campath® or the chimeric or humanized forms of 4B10. Due to the low dose used, only a modest level of depletion of B cells was observed in the spleen. On a per animal basis it appears that the humanized 4B10 antibody is as good or slightly better than Campath® at mediating lymphocyte
20 depletion. FIGS. 22A-22C show the level of CD4 T cells, CD8 T cells and CD19 B cells in the blood 72 hours after dosing with the chimeric and humanized antibodies.

Example 20: Relative Binding Efficiency of Anti-human CD52 Antibodies

[0446] The EC₅₀ values of selected anti-CD52 antibodies were estimated using CHO cells engineered to express CD52. CHO-CD52 cells were trypsinized in 0.25% trypsin, collected, and
25 rinsed with PBS/5% FBS. Cells were then deposited into round-bottom 96 well plates at 1E5 cells per well. Primary antibody staining was done with a 12 point serial dilution (1:2) of each anti-CD52 chimeric antibody starting at 50 µg/mL. FITC-conjugated goat FAB2 fragment of anti-human Fc gamma at 10 µg/mL (Jackson 109-096-098) secondary was used. Cells were washed 3 times in ice-cold PBS/5% FBS before and after each incubation. Cells were fixed
30 with PBS containing 2% methanol-free paraformaldehyde and evaluated by flow cytometry. The flow cytometry data was analyzed using Graph pad Prism software to determine EC₅₀ value with 95% confidence interval.

[0447] Binding data (FIG. 23) indicates that the new CD52 antibodies not only have different epitope specificities as mentioned earlier, but also have different binding characteristics as shown in the table given below. Campath-1H®, 7F11, 4B10, 2C3 and 12G6 chimeric antibodies showed relatively similar EC₅₀ values between 0.5 to 2.5 µg/ml. 9D9 chimeric antibody showed slightly different binding characteristics with EC₅₀ value around 5 to 7 µg/ml. 4B10 humanized antibody showed similar binding characteristics as that of chimeric 4B10 antibody, indicating that the humanized antibody retained the binding characteristics as that of chimeric 4B10 antibody.

Table 9: EC₅₀ (µg/mL)

Clone ID	Mean	STDEV
C1H*	1.36	0.46
2C3-Chi	1.32	0.33
4B10-Chi	2.18	0.33
4B10-H1/K1	2.23	0.50
7F11-Chi	2.22	0.29
9D9-Chi	6.05	1.18
12G6-Chi	0.95	0.21

* C1H refers to Campath-1H®.

Example 21: Humanization of Anti-CD52 Antibody Clone 7F11

[0448] Humanization of anti-human CD52 antibody clone 7F11 was performed by grafting the CDR regions from the mouse 7F11 antibody into a human antibody variable region framework as described in Example 14 for 4B10 antibody humanization. CDR-1, CDR-2, and CDR-3 sequences of the heavy chain and light chain of 7F11 were grafted into VH3-72 and VK2 A18b human framework regions, respectively. The human JH6 (WGQGTTTVTVSS: SEQ ID NO: 133) and JK2 (FGQGTEKLEIK: SEQ ID NO: 134) sequences were selected as the C-terminal peptides for the humanized heavy and light chains, respectively, to generate humanized heavy chain (7F11-SFD1 and 7F11-SFD2) and humanized light chain (7F11-VK2) variable region sequences for 7F11 (FIG. 24). The two humanized heavy chain variable region sequences (7F11-SFD1 and 7F11-SFD2) differ by one amino acid residue in the CDR-3 region.

The 7F11-SFD1 version has a threonine at position 93 (denoted by the Kabat numbering system), while the 7F11-SFD2 version has an alanine at this position. Position 93 is underlined for both 7F11-SFD1 and 7F11-SFD2 in FIG. 24.

[0449] The full-length heavy chain amino acid sequence of 7F11-SFD1 (SEQ ID NO: 274) and the full-length light chain amino acid sequence of 7F11-K2 (SEQ ID NO: 275) are shown in FIG. 107.

Example 22: Assessment of the Binding Activities of Chimeric and Humanized 7F11 Monoclonal Antibodies

[0450] Chimeric and humanized 7F11 antibodies (7F11-SFD1/K2 and 7F11-SFD2/K2) were produced and purified using the methods described in Example 3, and analyzed for their ability to bind to CD52 expressed on the surface of CHO-CD52 cells (CHO cells engineered to express human CD52) by flow cytometry. Briefly, 2×10^5 CHO-CD52 cells were incubated with an antibody at 10 µg/ml in PBS containing 5% fetal bovine serum and 5% goat serum. Bound antibody was detected with a FITC-labeled goat anti-human secondary antibody which detected chimeric or humanized anti-CD52 antibodies. Labeled cells were analyzed using a FACSCalibur system (Becton Dickinson) and the data was analyzed using FlowJo version 7.2 software (Tree Star, Inc, Oregon, USA). The histogram in FIG. 25 compares the levels of CD52 detected with chimeric and humanized 7F11 antibodies. The results indicate that the humanized 7F11 antibodies bound as well or slightly better than the chimeric 7F11 antibody to CD52 expressing cells.

Example 23: Humanization of Anti-CD52 Antibody Clone 2C3

[0451] Humanization of anti-human CD52 antibody clone 2C3 was performed by grafting the CDR regions from the mouse 2C3 antibody into a human antibody variable region framework as described in Example 14 for clone 4B10 antibody humanization. CDR-1, CDR-2, and CDR-3 sequences of the heavy chain and light chain of 2C3 were grafted into VH3-72 and VK2 A18b human framework regions, respectively. The human JH6 (WGQGTTVTVSS: SEQ ID NO: 133) and JK5 (FGQGTRLEIK: SEQ ID NO: 135) sequences were selected as the C-terminal peptides for the humanized heavy and light chains, respectively, to generate humanized heavy chain (2C3-SFD1) and light chain (2C3-VK1) variable region sequences for 2C3 (FIGS. 26A and B). Unlike humanized clones 4B10 and 7F11, the binding affinity for the CDR-grafted humanized 2C3 antibody was greatly reduced. Binding affinity was restored by introducing

- back mutations to the CDR-grafted structure, with the aim of limiting the number of back mutations to a minimum to keep the reshaped antibody as “human” as possible, thus reducing the possibility of immunogenicity. Single or multiple back mutations were incorporated into both the humanized heavy and light chain variable region sequences. The positions of the back mutations (as denoted by the Kabat numbering system) are depicted in Table 10 and Table 11 below. Antibodies generated with these back mutations were evaluated for restored binding affinity. Three light chain variants (2C3-VK1(L46R), also referred to as 2C3-VK11; 2C3-VK1(Y36L-L46R), also referred to as 2C3-VK12; and 2C3-VK1(M4I-A19V-Y36L-Q45K-L46R), also referred to as 2C3-VK13) and 5 heavy chain variants (2C3-SFD1(L78V), also referred to as 2C3-VH12; 2C3-SFD1(G49A), also referred to as 2C3-VH15; 2C3-SFD1(G49A-L78V), also referred to as 2C3-VH16; 2C3-SFD1(L18M-G49A-L78V), also referred to as 2C3-VH17; and 2C3-SFD1(L18M-G42E-G49A-L78V), also referred to as 2C3-VH19) were generated using standard molecular biology techniques. The amino acid sequences for CDR-grafted heavy chain variable region sequence 2C3-SFD1 and back mutants 2C3-VH12, 2C3-VH15, 2C3-VH16, 2C3-VH17, and 2C3-VH19 are shown in FIG. 26A with the back mutated amino acids underlined and the CDRs boldfaced. Similarly, for the light chain sequences, CDR-grafted variable region sequence 2C3-VK1 and back mutants 2C3-VK11, 2C3-VK12, and 2C3-VK13 are shown in FIG. 26B with the back mutated amino acids underlined and the CDRs boldfaced.
- [0452] The full-length heavy chain amino acid sequence of 2C3-SFD1 (SEQ ID NO: 272) and the full-length light chain amino acid sequence of 2C3-K12 (SEQ ID NO: 273) are shown in FIG. 106.

Table 10: 2C3 clone heavy chain back mutants

Clone ID	Mutation (Kabat numbering position)
2C3-VH12	L to V (78)
2C3-VH15	G to A (49)
2C3-VH16	G to A (49), L to V (78)
2C3-VH17	L to M (18), G to A (49), L to V (78)
2C3-VH19	L to M (18), G to E (42), G to A (49), L to V (78)

Table 11: 2C3 clone light (kappa) chain back mutants

Clone ID	Mutation (Kabat numbering position)
2C3-VK11	L to R (46)
2C3-VK12	Y to L (36) and L to R (46)
2C3-VK13	M to I (4), A to V (19), Y to L (36), QL to KR (45,46)

Example 24: Assessment of the Binding Activities of Chimeric and Humanized 2C3 Monoclonal Antibodies

5 [0453] Chimeric and humanized 2C3 antibodies were produced and purified using the methods described in Example 3. A number of the humanized antibodies produced by pairing heavy chain variants with light chain variants, and a corresponding chimeric antibody, were analyzed by flow cytometry for their ability to bind to CD52 expressed on the surface of CHO-CD52 cells, using the methods described in Example 22. The binding data suggest that clones
10 generated by pairing heavy chain variants with light chain variants 2C3-VK1 or 2C3-VK11 had reduced binding ability, while clones generated by pairing heavy chain variants with 2C3-VK12 or 2C3-VK13 showed binding equivalent to or better than that of a chimeric 2C3 antibody. A representative histogram of selected clones (FIG. 27A) compares the level of CD52 detected by chimeric and humanized 2C3 antibodies. Binding of 2C3-SFD1/K1 is reduced significantly
15 compared to that of the corresponding chimeric antibody. Incorporating a single mouse residue at position 46 (leucine to arginine) in the light chain (resulting in 2C3-VK11) did not restore the binding when paired with heavy chain 2C3-SFD1 to make antibody 2C3-SFD1/K11. Further, binding was not restored by incorporating three back mutations in the heavy chain (resulting in 2C3-VH17) to make antibody 2C3-H17/K11. However, binding was completely restored when
20 the 2C3-SFD1 heavy chain was paired with 2C3-VK12, which has two back mutations, to make antibody 2C3-SFD1/K12, suggesting that specific back mutations need to be incorporated to restore binding avidity. FIG. 27B shows a histogram of selected humanized clones that demonstrate binding equivalent to that of a chimeric 2C3 antibody. These results indicate that the back mutation of two amino acid residues in the 2C3-VK12 light chain was sufficient to
25 completely restore antibody avidity. The changes at residues 36 (Y to L) and 46 (L to R) were able to restore binding when paired with almost any heavy chain variant. As such, the humanized 2C3 clone showing restored binding with minimal framework residues derived from the original mouse antibody is 2C3-SFD1/K12.

Example 25: Humanization of Anti-CD52 Antibody Clone 12G6.

30 [0454] Humanization of anti-human CD52 antibody clone 12G6 was performed by grafting the CDR regions from the mouse 12G6 antibody into a human antibody variable region framework as described in Example 14 for clone 4B10 antibody humanization. CDR-1, CDR-2,

and CDR-3 sequences of the heavy chain and light chain of 12G6 were grafted into VH3-72 and VK2 A18b human framework regions, respectively. The human JH6 (WGQGTTTVTVSS: SEQ ID NO: 133) and JK2 (FGQGTKLEIK: SEQ ID NO: 134) sequences were selected as the C-terminal peptides for the humanized heavy and light chains, respectively, to generate humanized heavy chain (12G6-SFD1) and light chain (12G6-VK1) variable region sequences for 12G6 (FIGS. 28A and 28B). When the 12G6-SFD1 heavy chain variable region and 12G6-VK1 light chain variable region were combined in the humanized 12G6-SFD1/K1 antibody, the binding affinity for CD52 was greatly reduced. Binding affinity was restored by introducing back mutations to the CDR grafted structure. Single or multiple back mutations were incorporated into both the humanized heavy and light chain variable region sequences. The positions of these back mutations (as denoted by the Kabat numbering system) are depicted in Table 12 and Table 13 below. Antibodies generated with these back mutations were evaluated for restored binding affinity. Four light chain variants (12G6-VK1(Y36V), also referred to as 12G6-VK10; 12G6-VK1(Y36V-Q45K-L46R), also referred to as 12G6-VK11; 12G6-VK1(Y36V-L46R), also referred to as 12G6-VK12; and 12G6-VK1(L46R), also referred to as 12G6-VK13) and three heavy chain variants (12G6-SFD1(L78V), also referred to as 12G6-VH10; 12G6-SFD1(G49A), also referred to as 12G6-VH11; and 12G6-SFD1(G49A-L78V), also referred to as 12G6-VH12) were generated using standard molecular biology techniques. The amino acid sequences for the CDR grafted heavy chain variable region sequence 12G6-SFD1 and back mutants 12G6-VH10, 12G6-VH11, and 12G6-VH12 are shown in FIG. 28A with the back mutated amino acids underlined and the CDRs boldfaced. Similarly, for the light chain sequences, CDR grafted variable region sequence 12G6-VK1 and back mutants 12G6-VK10, 12G6-VK11, 12G6-VK12, and 12G6-VK13 are shown in FIG. 28B with the back mutated amino acids underlined and the CDRs boldfaced.

[0455] The full-length heavy chain amino acid sequence of 12G6-SFD1 (SEQ ID NO: 279) and the full-length light chain amino acid sequence of 12G6-K12 (SEQ ID NO: 280) are shown in FIG. 109.

Table 12: 12G6 clone heavy chain back mutants

Clone ID	Mutation (Kabat numbering position)
12G6-VH10	L to V (78)
12G6-VH11	G to A (49)
12G6-VH12	G to A (49) and L to V (78)

Table 13: 12G6 clone light (kappa) chain back mutants

Clone ID	Mutation (Kabat numbering position)
12G6-VK10	Y to V (36)
12G6-VK11	Y to V (36), QL to KR (45,46)
12G6-VK12	Y to V (36), L to R (46)
12G6-VK13	L to R (46)

Example 26: Assessment of the Binding Activities of Chimeric and Humanized 12G6**5 Monoclonal Antibodies**

[0456] Chimeric and humanized 12G6 antibodies were produced and purified using the methods described in Example 3. A number of the humanized antibodies produced by pairing heavy chain variants with light chain variants, and a corresponding chimeric antibody, were analyzed by flow cytometry for their ability to bind to CD52 expressed on the surface of CHO-
10 CD52 cells, using the methods described in Example 22. The binding data suggest that clones generated by pairing heavy chain variants with light chain variants 12G6-VK1, 12G6-VK10, or 12G6-VK13 had reduced binding ability, while clones generated by pairing heavy chain variants with 12G6-VK11 or 12G6-VK12 showed binding equivalent to or better than that of the corresponding chimeric 12G6 antibody. A representative histogram of selected clones (FIG. 29)
15 compares the level of CD52 detected by chimeric and humanized 12G6 antibodies. These results indicate that the back mutation of two amino acid residues in the 12G6 light chain variable region (clone 12G6-VK12) was sufficient to completely restore antibody specificity. The changes at Kabat numbering residues 36 (Y to V) and 46 (L to R) were able to restore binding when paired with almost any heavy chain variant. As such, the humanized 12G6 clone
20 showing restored binding with minimal framework residues derived from the original mouse antibody is 12G6-SFD1/K12.

Example 27: Humanization of Anti-CD52 Antibody Clone 9D9.

[0457] Humanization of anti-human CD52 antibody clone 9D9 was performed by grafting the CDR regions from the mouse 9D9 antibody into a human antibody variable region framework as
25 described in Example 14 for clone 4B10 antibody humanization. CDR-1, CDR-2, and CDR-3 sequences of the heavy chain and light chain of 9D9 were grafted into VH3-23 and VK2 A18b human framework regions, respectively. The human JH6 (WGQGTTVTSS: SEQ ID NO: 133) and JK2 (FGQGTELEIK: SEQ ID NO: 134) sequences were selected as the C-

terminal peptides for the humanized heavy and light chains, respectively, to generate humanized heavy chain (9D9-VH10) and light chain (9D9-VK2) variable region sequences (FIGS. 30A and 30B). When the 9D9-VH10 heavy chain and 9D9-VK2 light chain were combined in the humanized 9D9-H10/K2 antibody, the binding affinity for CD52 was greatly reduced. Binding

5 affinity was restored by introducing back mutations to the CDR grafted structure. Single or multiple back mutations were incorporated into both the humanized heavy and light chain variable region sequences. The positions of the back mutations (as denoted by the Kabat numbering system) are depicted in Table 14 and Table 15 below. Antibodies generated with these back mutations were evaluated for restored binding affinity. Four light chain variants

10 (9D9-VK2(Y36L-Q45K-L46R), also referred to as 9D9-VK12; 9D9-VK2(Y36L-L46R), also referred to as 9D9-VK13; 9D9-VK2(L46R), also referred to as 9D9-VK14; and 9D9-VK2(Q45K-L46R), also referred to as 9D9-VK15) and five heavy chain variants (9D9-VH10(W47L-V48T-S49A-N76S-L78V), also referred to as 9D9-VH11; 9D9-VH10(W47L-V48T-S49A), also referred to as 9D9-VH15; 9D9-VH10(W47L), also referred to as 9D9-VH16;

15 9D9-VH10(W47L-V48T), also referred to as 9D9-VH17; and 9D9-VH10(W47L-S49A), also referred to as 9D9-VH18) were generated using standard molecular biology techniques. The amino acid sequences for CDR-grafted heavy chain variable region sequence 9D9-VH10 and back mutants 9D9-VH11, 9D9-VH15, 9D9-VH16, 9D9-VH17, and 9D9-VH18 are shown in FIG. 30A with the back mutated amino acids underlined and the CDRs boldfaced. Similarly, for

20 the light chain sequences, CDR-grafted variable region sequence 9D9-VK2 and back mutants 9D9-VK12, 9D9-VK13, 9D9-VK14, and 9D9-VK15 are shown in FIG. 30B with the back mutated amino acids underlined and the CDRs boldfaced.

[0458] The full-length heavy chain amino acid sequences of 9D9-H16 (SEQ ID NO: 276) and 9D9-H18 (SEQ ID NO: 277), and the full-length light chain amino acid sequence of 9D9-K13

25 (SEQ ID NO: 278) are shown in FIG. 108.

Table 14: 9D9 heavy chain back mutants

Clone ID	Mutation (Kabat numbering position)
9D9-VH11	WVS to LTA (47-49), N to S (76), L to V (78)
9D9-VH15	WVS to LTA (47-49)
9D9-VH16	W to L (47)
9D9-VH17	WV to LT (47,48)
9D9-VH18	W to L (47) and S to A (49)

Table 15: 9D9 light (kappa) chain back mutants

Clone ID	Mutation (Kabat numbering position)
9D9-VK12	Y to L (36) and QL to KR (45,46)
9D9-VK13	Y to L (36) and L to R (46)
9D9-VK14	L to R (46)
9D9-VK15	QL to KR (45,46)

Example 28: Assessment of the Binding Activities of Chimeric and Humanized 9D9 Monoclonal Antibodies

- 5 [0459] Chimeric and humanized 9D9 antibodies were produced and purified using the methods described in Example 3. A number of the humanized antibodies produced by pairing heavy chain variants with light chain variants, and a corresponding chimeric antibody, were analyzed by flow cytometry for their ability to bind to CD52 expressed on the surface of CHO-CD52 cells (CHO cells engineered to express human CD52), using the methods described in
- 10 Example 22. The binding data suggest that clones generated by pairing heavy chain variants with light chain variants 9D9-VK2, 9D9-VK14, or 9D9-VK15 had reduced binding ability, while clones generated by pairing 9D9-VK12 or 9D9-VK13 light chain variants with back mutated heavy chain variants 9D9-VH11, 9D9-VH15, 9D9-VH16, and 9D9-VH18 showed binding equivalent to or better than that of the corresponding chimeric 9D9 antibody. When
- 15 light chain variants 9D9-VK12 and 9D9-VK13 were paired with the parental CDR grafted heavy chain 9D9-VH10 or the back mutated 9D9-VH17 sequence, binding was significantly reduced, suggesting that for humanized 9D9 clones, both heavy chain and light chain sequences have to be engineered with back mutations to restore binding ability. A representative histogram of selected clones (FIG. 31) compares the level of CD52 detected by chimeric and humanized 9D9
- 20 antibodies. These results indicate that the back mutation of two amino acid residues (e.g., Y to L at position 36, and L to R at position 46) in the 9D9 light chain variable region (clone 9D9-VK13) was necessary to restore antibody specificity when paired with heavy chains that were mutated at one position (e.g., W to L, at position 47) or at two positions (e.g., W to L at position 47 and S to A at position 49). As such, the humanized 9D9 clones showing restored binding
- 25 with minimal framework residues derived from the original mouse antibody are 9D9-H16/K13 and 9D9-H18/K13.

Example 29: Determination of Relative Binding Efficiency of Humanized Anti-Human CD52 Antibodies.

[0460] The EC₅₀ values of chimeric and humanized anti-CD52 antibodies were estimated using CD4+ T cells isolated from healthy donor PBMCs obtained from commercial sources (Bioreclimation, NY, USA). CD4+ T cells were isolated by negative selection using an EasySep kit (Stem Cell Technologies). CD4+ T cells isolated from huCD52 transgenic CD1 mouse spleen tissue were also used (Stem Cell Technologies) according to the methods described above in Example 20 for CHO-CD52 cells. Briefly, human CD4+ T cells were isolated from 50 ml of peripheral blood from healthy volunteers (Bioreclimation), and huCD52 transgenic mouse CD4+ T cells were isolated from spleen tissue. Cells were rinsed with PBS/5% FBS and deposited into round-bottom 96 well plates at 1×10^5 cells per well. Primary antibody staining was done with an 8 point serial dilution (1:3) of each anti-CD52 chimeric and humanized antibody starting at 100 µg/mL. A FITC-conjugated goat F(ab')₂ fragment of anti-human Fc gamma at 10 µg/mL (Jackson 109-096-098) secondary antibody was used. Cells were washed 3 times in ice-cold PBS/5% FBS before and after each incubation. Cells were fixed with PBS containing 2% methanol-free paraformaldehyde and evaluated by flow cytometry. The flow cytometry data was analyzed using GraphPad Prism software to determine an EC₅₀ value with 95% confidence interval. Based on the binding of anti-CD52 antibodies to CD4+ T cells isolated from human PBMCs and to CD4+ T cells isolated from spleen tissue of human CD52 transgenic mice, binding curves (FIGS. 32A, 32B, 32C) were generated and EC₅₀ values estimated and shown in FIG. 33. All of the antibodies showed similar binding characteristics to both human CD4+ T cells and to CD4+ T cells isolated from human CD52 transgenic mice. Binding data indicate that the humanized antibodies have equivalent or better binding affinities compared to their parental chimeric antibodies, suggesting that binding affinity is retained or improved upon humanization. Humanized 2C3 and 12G6 antibodies have at least two fold lower EC₅₀ values than a Campath-1H® antibody as determined by this cell binding assay.

Example 30: Evaluation of the Binding of Humanized Anti-CD52 Antibodies to a Defined Lymphocyte Population

[0461] Campath-1H® (C1H) and humanized 2C3 (2C3-SFD1/K12), 9D9 (9D9-H16/ K13 and 9D9-H18/K13), and 12G6 (12G6-SFD1/K11, 12G6-SFD1/K12) antibodies were evaluated for their binding to various PBMC subsets in normal donor PBMCs using the methods described

above in Example 7 for chimeric anti-CD52 antibodies. A number of fluorochrome conjugated antibodies were used for flow cytometric analysis. Anti-CD27-PE, anti-CD19 and anti-CD11c-PE Cy5, anti-CD56 and anti-CD123-PE Cy7, anti-CD16-APC Cy7, and CD4-APC were obtained from BD Biosciences (San Diego, CA), while anti-CD54RA-ECD and anti-HLA-DR-ECD were obtained from Beckman Coulter. Anti-CD3-Pacific Blue, anti-CD8 and anti-CD14-Pacific Orange, and anti-CD4-APC cy5.5 were obtained from Invitrogen (CA). All of the humanized anti-human CD52 antibodies (9D9-H18/K13, 9D9-H16/K13, 12G6-SFD1/K11, 12G6-SFD1/K12, and 2C3-SFD1/K12) as well as the Campath-1H® were conjugated to FITC. Healthy human peripheral blood mononuclear cells were obtained either from cryopreserved buffy coats or from mononuclear cells separated from the blood of normal donors obtained from commercial vendors (Bioreclamation, NY, USA) as described above in Example 7. For enrichment of mononuclear cells, human peripheral blood was diluted 1:1 with sterile phosphate buffered saline (PBS) and carefully layered over Ficoll-Hypaque (GE Healthcare Bio-Sciences, Uppsala, Sweden) and centrifuged for 30 min at room temperature. The interphase layer of mononuclear cells was drawn out and washed in PBS containing 5% fetal bovine serum (FACS buffer). Contaminating red blood cells (RBCs) were lysed with RBC lysing solution (Sigma, St. Louis, MO, USA). Cells were resuspended in cold FACS buffer and the debris was removed using a 40 µm filter. Multi color flow cytometry was performed to evaluate the binding ability of humanized anti-human CD52 antibodies 2C3 (2C3-SFD1/K12), 9D9 (9D9-H16/K13 and 9D9-H18/K13) and 12G6 (12G6-SFD1/K11 and 12G6-SFD1/K12) as compared to Campath-1H®.

[0462] Briefly, replicates of 1×10^6 PBMCs in FACS buffer were incubated with cocktails of pre-titrated dilutions of antibodies to examine either lymphocyte or myeloid derived cells. The lymphocyte cocktail comprised antibodies against CD3, CD27, CD45RA, CD56, CD19, CD8, CD4, and CD16. The antibody cocktail to define myeloid populations included antibodies against HLA-DR, CD11c, CD123, CD4, and CD14. In each of the cocktails, one of the anti-CD52 antibodies was included at 10 µg/ml concentration. The cells were stained for 30 min at 4°C and were washed and fixed in PBS containing 1% paraformaldehyde. 100,000 events of the stained cells were acquired on a BD LSR II flow cytometer (BD Biosciences, San Jose, CA), and the data was analyzed using FlowJo version 7.2 software (Tree Star, Inc, Oregon, USA). Multiple subsets with distinct phenotypic characteristics have been defined among B and T lymphocytes, and CD52 has been shown to be expressed on all human lymphocytes. Multi color

flow cytometry analysis was performed to identify the lymphocyte subsets, and to assess similarities and differences in the binding characteristics of the humanized anti-CD52 antibodies to cell surface CD52 on defined subsets. Using a combination of markers, phenotypically distinct cell populations corresponding to B, T, NK and antigen presenting cell lineages were first defined. The intensity of staining, which corresponds to the ability of humanized anti-CD52 antibodies to detect CD52 expression on each of the defined cell populations, was assessed and compared to that of Campath-1H®. The histograms (FIG. 34) compare the level of CD52 detected by each antibody on individual populations. The results indicate that all of the humanized anti-CD52 antibodies bind to cell surface CD52 to a similar extent. Further, no differences were observed between Campath-1H® and humanized anti-CD52 antibodies with respect to the level of detection of cell surface CD52. Analysis was performed on six different donors. Representative data generated using cells derived from one donor is shown in FIG. 34. A similar binding pattern was observed with cells from other donors.

Example 31: Assessment of the ADCC Activities of Chimeric and Humanized 7F11

Monoclonal Antibodies

[0463] Humanized and chimeric 7F11 antibodies were evaluated for their ability to mediate ADCC killing of CD52 expressing cells. An ADCC assay was carried out using the methods described above in Example 6. Briefly, CHO K1 cells engineered to express CD52 protein (CHO-CD52) were used as target cells. The target cells were labeled with $\text{Na}_2^{51}\text{CrO}_4$ (New England Nuclear, Boston, MA) at 37°C for 2-3 hrs. The cells were washed, re-suspended in RPMI 1640 media with 10% FCS, and mixed with an IgG control antibody, a chimeric 7F11 antibody, or a humanized 7F11 antibody (7F11-SFD1/K2 or 7F11-SFD2/K2) at various concentrations ranging from 5 µg/ml to 0.01 µg/ml. Human NK cells isolated from PBMCs using an NK cell isolation kit (Stem Cell Technologies) were used as effector cells and were added at a 1:5 target to effector cell ratio. After 2-6 hrs incubation, 25 µl of cell-free supernatant were collected from each well and counted in a MicroBeta Trilux Scintillation Counter (Wallac, Gaithersburg, MD). The amount of ^{51}Cr spontaneously released was obtained by incubating target cells in medium alone. Spontaneous release from target cells was typically less than 20%. The total amount of ^{51}Cr incorporated was determined by adding 1% Triton X-100 in distilled water, and the percentage lysis was calculated as follows: $[(\text{sample c.p.m.} - \text{spontaneous c.p.m.}) / (\text{total c.p.m.} - \text{spontaneous c.p.m.})] \times 100$. FIG. 35 illustrates the concentrations of

control IgG, chimeric 7F11 antibody, and humanized 7F11 antibodies used in the assay (X axis) vs. % specific lysis (Y axis). The results indicate that humanized 7F11 antibodies (7F11-SFD1/K2 and 7F11-SFD2/K2) mediated equivalent or slightly better ADCC killing as compared to a chimeric 7F11 antibody. The control IgG1 isotype showed only low levels of background killing at the concentrations tested.

Example 32: Assessment of the CDC Activities of Chimeric and Humanized 7F11 Monoclonal Antibodies

[0464] Humanized and chimeric 7F11 antibodies were evaluated for their ability to mediate complement dependent cytotoxicity (CDC) of CD52 expressing cells. A CDC assay was carried out using the methods described above in Example 5 for chimeric anti-CD52 antibodies. Briefly, CHO K1 cells engineered to express CD52 protein (CHO-CD52) were used as target cells and labeled with $\text{Na}_2^{51}\text{CrO}_4$ (New England Nuclear, Boston, MA) at 37°C for 2-3 hrs. The cells were washed, resuspended in RPMI 1640 media, and mixed with an IgG control antibody, a chimeric 7F11 antibody, or a humanized 7F11 antibody (7F11-SFD1/K2 or 7F11-SFD2/K2) at various concentrations ranging from 20 µg/ml to 500 ng/ml. Human complement (Sigma) was added to the experimental wells to a final concentration of 10%. After a 1-5-hour incubation, 25 µl of cell-free supernatant were collected from each well and counted in a MicroBeta Trilux Scintillation Counter (Wallac, Gaithersburg, MD). The amount of ^{51}Cr spontaneously released was obtained by incubating target cells in medium alone. Spontaneous release from target cells was typically less than 20%. The total amount of ^{51}Cr incorporated was determined by adding 1% Triton X-100 in distilled water, and the percentage lysis was calculated as follows: [(sample counts per minute (c.p.m.) - spontaneous c.p.m.)/(total c.p.m.-spontaneous c.p.m.)] X 100. FIG. 36 illustrates the concentrations of control IgG, chimeric 7F11 antibody, and humanized 7F11 antibodies (7F11-SFD1/K2 and 7F11-SFD2/K2) used in the assay (X axis) vs. % specific lysis (Y axis). The results indicate that the chimeric 7F11 antibody and humanized antibody 7F11-SFD1/K2 mediated equivalent killing, while humanized antibody 7F11-SFD2/K2 mediated significantly better CDC killing than the chimeric 7F11 antibody. The control IgG1 isotype antibody showed only low levels of background killing at the concentrations tested.

Example 33: Assessment of the ADCC Activities of Chimeric and Humanized 2C3 Monoclonal Antibodies

[0465] Humanized and chimeric 2C3 antibodies were evaluated for their ability to mediate ADCC killing of CD52 expressing cells. An ADCC assay was carried out using the methods described above in Example 6, with slight modifications. Briefly, T cells isolated from healthy donor PBMCs using a CD4+ T cell isolation kit (Stem Cell Technologies) were used as target cells. The target cells were labeled overnight with $\text{Na}_2^{51}\text{CrO}_4$ (New England Nuclear, Boston, MA) at 37°C. The cells were washed, re-suspended in RPMI 1640 media with 10% FCS, and mixed with an IgG control antibody, a chimeric 2C3 antibody, or a humanized 2C3 antibody (2C3-SFD1/K12) at various concentrations ranging from 10 µg/ml to 100 pg/ml. Human NK cells isolated from PBMCs (using an NK cell isolation kit from Stem Cell Technologies) were used as effector cells and were added at a 1:5 target to effector cell ratio. After 2-6 hrs of incubation, 25 µl of cell-free supernatant were collected from each well and counted in a MicroBeta Trilux Scintillation Counter (Wallac, Gaithersburg, MD). The amount of ^{51}Cr spontaneously released was obtained by incubating target cells in medium alone. Spontaneous release from target cells was typically less than 20%. The total amount of ^{51}Cr incorporated was determined by adding 1% Triton X-100 in distilled water, and the percentage lysis was calculated as follows: $[(\text{sample c.p.m.} - \text{spontaneous c.p.m.}) / (\text{total c.p.m.} - \text{spontaneous c.p.m.})] \times 100$. FIG. 37 illustrates the concentrations of control IgG, chimeric 2C3 antibody, and humanized 2C3 antibody (2C3-SFD1/K12) used in the assay (X axis) vs. % specific lysis (Y axis). The results indicate that the humanized 2C3 antibody 2C3-SFD1/K12 mediated ADCC killing equivalent to that of the 2C3 chimeric antibody. The IgG1 isotype control showed only low levels of background killing at the concentrations tested.

Example 34: Assessment of the CDC Activities of Chimeric and Humanized 2C3 Monoclonal Antibodies

[0466] Humanized and chimeric 2C3 antibodies were evaluated for their ability to mediate complement dependent cytotoxicity (CDC) of CD52 expressing cells. A CDC assay was carried out using the methods described above in Example 5, with slight modifications. Briefly, T cells isolated from healthy donor PBMCs were used as target cells and labeled overnight with $\text{Na}_2^{51}\text{CrO}_4$ (New England Nuclear, Boston, MA) at 37°C. After overnight labeling, the cells were washed, re-suspended in RPMI 1640 media with 10% FCS, and mixed with an IgG control

antibody, a chimeric 2C3 antibody, or a humanized 2C3 antibody (2C3-SFD1/K12) at various concentrations ranging from 10 µg/ml to 10 ng/ml. Human complement (Sigma) was added to the experimental wells to a final concentration of 10%. After a 1-5 hour incubation, 25 µl of cell-free supernatant were collected from each well and counted in a MicroBeta Trilux

- 5 Scintillation Counter (Wallac, Gaithersburg, MD). The amount of ⁵¹Cr spontaneously released was obtained by incubating target cells in medium alone. Spontaneous release from target cells was typically less than 20%. The total amount of ⁵¹Cr incorporated was determined by adding 1% Triton X-100 in distilled water, and the percentage lysis was calculated as follows: [(sample counts per minute (c.p.m.) - spontaneous c.p.m.)/(total c.p.m.-spontaneous c.p.m.)] X 100. FIG. 10 38 illustrates the concentrations of control IgG, chimeric 2C3 antibody, and humanized 2C3 antibody (2C3-SFD1/K12) used in the assay (X axis) vs. % specific lysis (Y axis). The results indicate that the chimeric 2C3 antibody and the humanized 2C3 antibody (2C3-SFD1/K12) mediated equivalent lysis. The control IgG1 isotype antibody showed only low levels of background killing at the concentrations tested.

15 **Example 35: Assessment of the ADCC Activities of Chimeric and Humanized 12G6 Monoclonal Antibodies**

- [0467] Humanized and chimeric 12G6 antibodies were evaluated for their ability to mediate ADCC killing of CD52 expressing cells. An ADCC assay was carried out by chromium release assays using T cells isolated from healthy donor PBMCs as target cells, as described above in 20 Example 31. FIG. 39 illustrates the concentrations of control IgG, chimeric 12G6 antibody, and humanized 12G6 antibodies (12G6-SFD1/K11 or 12G6-SFD1/K12) used in the assay (X axis) vs. % specific lysis (Y axis). The results indicate that humanized 12G6 antibodies 12G6-SFD1/K11 and 12G6-SFD1/K12 mediated equivalent ADCC killing as compared to the 12G6 chimeric antibody. The IgG1 isotype control showed only low levels of background killing at 25 the concentrations tested.

Example 36: Assessment of the CDC Activities of Chimeric and Humanized 12G6 Monoclonal Antibodies

- [0468] Humanized and chimeric 12G6 antibodies were evaluated for their ability to mediate complement dependent cytotoxicity (CDC) of CD52 expressing cells. A CDC assay was carried 30 out by chromium release assays using T cells isolated from healthy donor PBMCs as target cells, as described above in Example 32. FIG. 40 illustrates the concentrations of control IgG,

chimeric 12G6 antibody, and humanized 12G6 antibodies (12G6-SFD1/K11 and 12G6-SFD1/K12) used in the assay (X axis) vs. % specific lysis (Y axis). The results indicate that the chimeric 12G6 antibody mediated equivalent lysis as compared to humanized 12G6 antibodies (12G6-SFD1/K11 and 12G6-SFD1/K12). The control IgG1 isotype antibody showed only low levels of background killing at the concentrations tested.

Example 37: Assessment of the ADCC Activities of Chimeric and Humanized 9D9 Monoclonal Antibodies

[0469] Humanized and chimeric 9D9 antibodies were evaluated for their ability to mediate ADCC killing of CD52 expressing cells. An ADCC assay was carried out by chromium release assays using T cells isolated from healthy donor PBMCs as target cells, as described above in Example 31. FIG. 41 illustrates the concentrations of control IgG, chimeric 9D9 antibody, and humanized 9D9 antibodies (9D9-H10/K13, 9D9-H11/K13, 9D9-H16/K13, and 9D9-H18/K13) used in the assay (X axis) vs. % specific lysis (Y axis). The results indicate that the chimeric and humanized 9D9 antibodies (with the exception of 9D9-H10/K13) mediated equivalent ADCC killing. The IgG1 isotype control showed only low levels of background killing at the concentrations tested.

Example 38: Assessment of the CDC Activities of Chimeric and Humanized 9D9 Monoclonal Antibodies

[0470] Humanized and chimeric 9D9 antibodies were evaluated for their ability to mediate complement dependent cytotoxicity (CDC) of CD52 expressing cells. A CDC assay was carried out by chromium release assays using T cells isolated from healthy donor PBMCs as target cells, as described above in Example 32. FIG. 42 illustrates the concentrations of control IgG, chimeric 9D9 antibody, and humanized 9D9 antibodies (9D9-H10/K13, 9D9-H11/K13, 9D9-H16/K13, and 9D9-H18/K13) used in the assay (X axis) vs. % specific lysis (Y axis). The results indicate that a chimeric 9D9 antibody mediated equivalent lysis as compared to humanized 9D9 antibodies (with the exception of 9D9-H10/K13). The control IgG1 isotype antibody showed only low levels of background killing at the concentrations tested.

Example 39: Assessment of the ADCC Activities of Campath-1H® and Humanized Anti-CD52 antibodies on primary T cells

[0471] Campath-1H® and humanized anti-CD52 antibodies were evaluated for their ability to mediate ADCC killing of CD52 expressing cells. An ADCC assay was carried out by chromium

release assays using T cells isolated from healthy donor PBMCs as target cells, as described above in Example 31. FIG. 43 illustrates the concentrations of control IgG, Campath-1H®, and humanized 2C3-SFD1/K12, 9D9-H16/K13, 9D9-H18/K13, 12G6-SFD1/K11, and 12G6-SFD1/K12 antibodies used in the assay (X axis) vs. % specific lysis (Y axis). The results indicate that the above humanized 2C3, 9D9, and 12G6 antibodies mediated ADCC killing equivalent to that of Campath-1H® at concentrations in excess of 10 ng/ml. The IgG1 isotype control showed only low levels of background killing at the concentrations tested.

Example 40: Assessment of the CDC Activities of Campath-1H® and Humanized Anti-CD52 antibodies on primary T cells

[0472] Campath-1H® and humanized anti-CD52 antibodies were evaluated for their ability to mediate complement dependent cytotoxicity (CDC) of CD52 expressing cells. A CDC assay was carried out by chromium release assays using T cells isolated from healthy donor PBMCs as target cells, as described above in Example 32. FIG. 44 illustrates the concentrations of control IgG, Campath-1H®, and humanized 2C3-SFD1/K12, 9D9-H16/K13, 9D9-H18/K13, 12G6-SFD1/K11, and 12G6-SFD1/K12 antibodies used in the assay (X axis) vs. % specific lysis (Y axis). The results indicate that humanized 2C3 and 12G6 antibodies mediated CDC killing equivalent to Campath-1H®, while humanized 9D9 antibodies demonstrated significantly reduced CDC activity, similar to their corresponding chimeric antibody. The IgG1 isotype control showed only low levels of background killing at the concentrations tested.

Example 41: Assessment of Neutralizing Ability of Serum Samples Containing Anti-Campath-1H® Neutralizing Antibodies to Block Humanized 2C3, 12G6, and 9D9 Anti-CD52 Antibody Activity

[0473] To assess the ability of humanized antibodies to bind to CD52 expressing cells in the presence of neutralizing antibodies against Campath-1H®, anti-CD52 antibodies (Campath-1H®, 2C3-SFD1/K12, 9D9-H16/K13 and 12G6-SFD1/K12) were reacted with human serum containing anti-Campath-1H® antibody reactivity and evaluated for binding to CD52 expressing Raji cells. Serum samples obtained from relapsing remitting multiple sclerosis patients who were enrolled in the CAMMS223 study (The CAMMS223 Trial Investigators, "Alemtuzumab vs. interferon Beta-1a in early multiple sclerosis," *N Engl J Med* 359:1786-1801 (2008)) were used in the assay. Repeated administration of the Campath-1H® antibody resulted in generation of anti-Campath-1H® antibody responses in most patients. The anti-Campath-1H antibody titer

is very low at month 12 in most patients, and increased significantly upon administration of a second cycle of Campath-1H® resulting in a high titer anti-Campath-1H® response in the sera at month 13. Anti-CD52 antibody neutralization assays were carried out using month 12 and month 13 serum samples obtained from five different MS patients (MS-1 to MS-5) who had

5 been treated with Campath-1H® under the CAMMS223 protocol. FITC-conjugated anti-CD52 antibodies Campath-1H®, 2C3-SFD1/K12, 12G6-SFD1/K12, and 9D9-H16/K13 (used in Example 30 and shown to bind to CD52 expressing cells) were used to stain Raji cells that express human CD52 in the absence or presence of a range of dilutions of serum obtained from patients who have been treated with Campath-1H. Briefly, MS patient serum samples (month

10 12 and month 13) were made into 6 fold serial dilutions and incubated with 10 µg/ml of FITC-conjugated anti-CD52 antibodies (Campath-1H®, 2C3-SFD1/K12, 12G6-SFD1/K12, and 9D9-H16/K13) for 1 hr at 37°C. Raji cells were rinsed with a staining buffer containing HBSS, 5% FBS, and 0.1% azide, and then deposited into round-bottom 96 well plates at 1×10^5 cells per well. Cells were blocked with 10 µg/ml of human IgG Fc fragment for 30 min on ice in staining

15 buffer. The cells were then washed with staining buffer and re-suspended in 100 µl of the antibody-serum mix as described above. After 30 minutes on ice, cells were washed and fixed with BD Cytofix and the FITC-labeled antibody coated cells were analyzed using a FACSCalibur system (Becton Dickinson), after which the data was analyzed using FlowJo version 7.2 software (Tree Star, Inc, Oregon, USA). Binding of FITC-conjugated anti-CD52

20 antibodies in the presence of anti-Campath-1H® neutralizing antibodies in the serum was assessed by flow cytometry and % binding relative to control, as a measure of inhibition was calculated as $(\text{MFI with serum} / \text{MFI control (no serum)}) \times 100$. Representative data from one of the donors (MS-1) is shown in FIG. 45. The X axis denotes the serum dilution factor and the Y axis denotes the % control binding as a measure of antibody neutralizing activity. The data

25 clearly demonstrate that month 12 serum samples have no inhibitory effect on Campath-1H® or other anti-CD52 antibodies, suggesting that there are low or no anti-Campath-1H® blocking antibodies in the serum. Month 13 serum samples mediated complete inhibition of Campath-1H® binding even at a 1:1000 dilution of serum, but did not mediate inhibition of 2C3, 12G6, and 9D9 humanized anti-CD52 antibodies even at the highest concentration (1:24 dilution)

30 tested. Two of the five patients developed anti-Campath-1H® neutralizing antibody titers of >1:1000, whereas three other patients had about 1:100 Campath-1H® neutralizing antibody titers. Even though two of the patients' month 13 sera had relatively high neutralizing antibody

titers of >1:1000 against Campath-1H®, these sera did not inhibit binding of humanized 2C3-SFD1/K12, 12G6-SFD1/K12, and 9D9-H16 /K13 antibodies, suggesting that the anti-Campath-1H® antibody reactivity in patients treated with Campath-1H® did not block binding of these humanized antibodies to CD52 as presented on cells.

5 **Example 42: Analysis of Depletion and Repopulation of Anti-CD52 Antibodies in huCD52 Transgenic Mice (4B10-H1/K1)**

[0474] The depleting activities of Campath-1H® and the humanized anti-CD52 antibody (4B10-H1/K1) at different dose levels were examined in the huCD52 transgenic mouse. Mice were injected intravenously with 0.1, 0.5, 1.0 or 5.0 mg/kg of each antibody. Two hours post dosing, serum was collected to examine the level of circulating cytokines. Three days post
10 dosing, mice were sacrificed, and blood and spleens were collected from each mouse (N=5) to determine the level of cell depletion using flow cytometry analysis. Samples were evaluated to determine the relative numbers of total T helper cell (CD4+), cytotoxic T cell (CD8+), B cell (B220+) and myeloid cell subpopulations present in the circulating peripheral blood or spleen of
15 huCD52 transgenic mice. In addition, T and B cell subset analysis was performed to determine the overall depleting effect. A subset of mice (N=5) were kept alive to monitor the repopulation kinetics. Depletion was greatest in the T cell compartment with CD4+ T cells being depleted most followed by CD8+ T cells, B cells, NK cells, and other myeloid cells. Within the CD4+ T cell compartment, naïve CD4+ T cells were depleted the most followed by CD4+ central
20 memory (CM), CD4+ effector memory (EM), and CD4+ regulatory T cells (Treg). A similar pattern was observed for CD8+ T cells (Naïve>CM>EM). Conversely, mature B cells were depleted to a greater extent than immature B cells. Comparison of Campath-1H® treated mice to 4B10-H1/K1 treated mice demonstrated similar patterns of cells in both the blood and spleen at each of the doses examined.

25 [0475] Serum cytokine analysis demonstrated dose dependent increases for TNFα, IL-6 and MCP-1. The circulating level of these cytokines remained elevated compared to untreated mice at the 0.5 and 0.1 mg/kg doses as well. Slight increases were also observed for IL-10 in the Campath-1H® treated group at the three highest doses but only for the highest dose of the humanized 4B10-H1/K1 treated group. No significant increases in the level of circulating IL-12
30 or IFNγ (not shown) were noted.

[0476] By 50-60 days post dosing, with the exception of the 1.0 mg/kg group, lymphocyte levels in all of the Campath-1H® dosed groups had rebounded to the levels of untreated mice. In the 1.0 mg/kg group, lymphocytes had returned to normal levels by 80 days post dosing.

Similar repopulation kinetics were also observed for the humanized 4B10-H1/K1 antibody

5 treated mice. Lymphocytes had rebounded to control levels by 50 days post dosing in all 4B10-H1/K1 treated groups with the exception of the 0.5 mg/kg level. Levels of circulating lymphocytes in the 0.5 mg/kg group remained decreased throughout the course of the monitoring period. Total lymphocytes were monitored for repopulation in the blood.

[0477] FIGS. 46A-46E show the level of CD4+ T cells, CD8+ T cells and B220+ B cells in

10 the blood 72 hours after dosing with Campath-1H® ("Campath") and humanized 4B10-H1/K1 ("4B10") antibodies. FIGS. 47A-47E show the level of CD4+ T cells, CD8+ T cells and B220+ B cells in the spleen 72 hours after dosing with Campath-1H® ("Campath") and humanized 4B10-H1/K1 ("4B10") antibodies. FIGS. 48A-48E show the levels of circulating cytokines 2

15 hours after dosing with Campath-1H® ("Campath") and humanized 4B10-H1/K1 ("4B10") antibodies. FIGS. 49A-49B show the repopulation of circulating lymphocytes over a timecourse after dosing with Campath-1H® ("Campath") and humanized 4B10-H1/K1 ("4B10") antibodies.

Example 43: Analysis of Depletion and Repopulation of Anti-CD52 Antibodies in huCD52 Transgenic Mice (7F11-SFD1/K2 and 7F11-SFD2/K2)

[0478] The depleting activity of humanized antibodies (7F11-SFD1/K2 and 7F11-SFD2/K2) at

20 different dose levels was examined in huCD52 transgenic mice. Mice were injected intravenously with 0.1, 0.5, 1.0 or 5.0 mg/kg of each antibody. Two hours post dosing, serum was collected to examine the level of circulating cytokines. Three days post dosing, mice were sacrificed, and blood and spleens were collected from each mouse (N=5) to determine the level of cell depletion using flow cytometry analysis. Samples were evaluated to determine the

25 relative numbers of total T helper cell (CD4+), cytotoxic T cell (CD8+), B cell (B220+) and myeloid cell subpopulations present in the circulating peripheral blood or spleen of huCD52 transgenic mice. In addition, T and B cell subset analysis was performed to determine the overall depleting effect. A subset of mice (N=5) were kept alive to monitor the repopulation kinetics. Administration of each humanized 7F11 antibody (7F11-SFD1/K2 and 7F11-SFD2/K2) at all

30 doses resulted in depletion of a significant number of both T cells and B cells in the blood. These data also demonstrated that various T and B cell subsets are depleted to differing degrees

depending on the dose of antibody used. Naïve T cells (both CD4 and CD8) demonstrated the most depletion with other cell populations (including memory and T reg cells) being depleted to a lesser degree. In the B cell compartment, mature B cells were depleted more readily than immature B cells. In the spleen, dose dependent depletion was observed with significant
5 depletion of lymphocytes being observed at the 5 and 1 mg/kg dose levels. Similar to the case with blood, naïve T cells were more readily depleted than memory cells. B cells were depleted to a lesser extent than T cells with each of the humanized 7F11 clones (7F11-SFD1/K2 and 7F11-SFD2/K2). Depletion was not observed for NK cells or neutrophils in the blood or the spleen at any of the doses injected. Serum cytokine analysis demonstrated dose dependent
10 increases for both TNF α and IL-6. Levels of these cytokines remained elevated compared to untreated mice at the 0.5 and 0.1 mg/kg doses as well. Dose dependent increases in the level of circulating MCP-1 were also noted.

[0479] By 30 days post dosing, lymphocyte levels in the 0.5 and 0.1 mg/kg dosed groups had rebounded to the levels of untreated mice. In the 1.0 and 5.0 mg/kg groups, lymphocytes had
15 returned to normal levels by 50 and 80 days, respectively, for clone 7F11-SFD1/K2 and by 80 days post dosing for both the 1.0 and 5.0 mg/kg groups of clone 7F11-SFD2/K2. Total lymphocytes were monitored for repopulation in the blood.

[0480] FIGS. 50A-50E show the level of CD4+ T cells, CD8+ T cells and B220+ B cells in the blood 72 hours after dosing with the humanized 7F11-SFD1/K2 ("7F11 SFD1") and
20 7F11-SFD2/K2 ("7F11 SFD2") antibodies. FIGS. 51A-51E show the level of CD4+ T cells, CD8+ T cells and B220+ B cells in the spleen 72 hours after dosing with the humanized 7F11-SFD1/K2 ("7F11 SFD1") and 7F11-SFD2/K2 ("7F11 SFD2") antibodies. FIGS. 52A-52F show the levels of circulating cytokines 2 hours after dosing with the humanized 7F11-SFD1/K2 ("7F11 SFD1") and 7F11-SFD2/K2 ("7F11 SFD2") antibodies. FIGS. 53A-53B show the
25 repopulation of circulating lymphocytes over a timecourse after dosing with the humanized 7F11-SFD1/K2 ("7F11 SFD1") and 7F11-SFD2/K2 ("7F11 SFD2") antibodies.

Example 44: Analysis of 7F11 Humanized Anti-CD52 Antibodies in CD52 Transgenic Mice (7F11-SFD1/K2 and 7F11-SFD2/K2)

[0481] The depleting activity of the chimeric 7F11 antibodies and humanized 7F11-SFD1/K2
30 and 7F11-SFD2/K2 antibodies in comparison to Campath-1H® was examined in the huCD52 transgenic mouse. Mice were injected intravenously with 1.0 mg/kg of each antibody. Three

days post dosing, mice were sacrificed, and blood and spleens were collected from each mouse (N=5) to determine the level of cell depletion using flow cytometry analysis. Samples were evaluated to determine the relative numbers of total T helper cell (CD4+), cytotoxic T cell (CD8+), B cell (B220+) and myeloid cell subpopulations present in the circulating peripheral blood or spleen of huCD52 transgenic mice. Administration of Campath-1H® resulted in depletion of a significant number of both T cells and B cells in the blood and spleen. Although a comparable level of T cell depletion was observed in the blood for both the chimeric and humanized 7F11 antibodies (7F11-SFD1/K2 and 7F11-SFD2/K2), B cells were depleted to a lesser extent. This observation was also apparent in the spleen, where significant T cell depletion was noted, but only a modest level of B cell depletion was achieved with the 7F11 antibodies (7F11-SFD1/K2 and 7F11-SFD2/K2).

[0482] FIGS. 54A-54B show the level of CD4+ T cells, CD8+ T cells and B220+ B cells in the blood 72 hours after dosing with Campath-1H® ("Campath"), 7F11-chimeric antibodies, and humanized 7F11-SFD1/K2 and 7F11-SFD2/K2 antibodies.

Example 45: Analysis of PK Profiles of Anti-CD52 Antibodies in CD52 Transgenic Mice (7F11-SFD1/K2 and 7F11-SFD2/K2)

[0483] To ensure that the humanization process did not alter the clearance rate of the antibody, the pharmacokinetic profile of the chimeric 7F11 anti-CD52 antibody and humanized 7F11-SFD1/K2 and 7F11-SFD2/K2 anti-CD52 antibodies was determined in huCD52 transgenic mice. Mice were injected intravenously with antibodies at 5 mg/kg and blood was collected at various timepoints beginning two hours post dosing. The circulating levels of each antibody were evaluated using an anti-human IgG ELISA. For each of the humanized clones, there was a slight difference in the Cmax noted at 2 hours post dosing. Clearance rates for the chimeric 7F11 antibody and humanized 7F11-SFD1/K2 and 7F11-SFD2/K2 antibodies were similar to each other as well as to Campath-1H® over the course of the experiment, indicating that the humanization process did not significantly alter the pharmacokinetic profile of the antibodies.

[0484] FIG. 55 shows the level of Campath-1H® ("Campath"), 7F11-chimeric antibody and humanized 7F11-SFD1/K2 and 7F11-SFD2/K2 antibodies in the blood over a timecourse after dosing.

Example 46: Analysis of Depletion and Repopulation of Anti-CD52 Antibodies in huCD52 transgenic mice (2C3-SFD1/K12)

[0485] The depleting activity of the 2C3-SFD1/K12 clone at different dose levels was examined in the huCD52 transgenic mouse. Mice were injected intravenously with 0.1, 0.5, 1.0 or 5.0 mg/kg of antibody. Two hours post dosing, serum was collected to potentially examine the level of circulating cytokines. Three days post dosing, mice were sacrificed, and blood and spleens were collected from each mouse (N=5) to determine the level of cell depletion using flow cytometry analysis. Samples were evaluated to determine the relative numbers of total T helper cell (CD4+), cytotoxic T cell (CD8+), B cell (B220+) and myeloid cell subpopulations present in the circulating peripheral blood or spleen of huCD52 transgenic mice. In addition, T and B cell subset analysis was performed to determine the overall depleting effect. A subset of mice (N=5) were kept alive to monitor the repopulation kinetics. Administration of 2C3-SFD1/K12 at the 5, 1, and 0.5 mg/kg doses resulted in depletion of a significant number of both T cells and B cells in the blood. A variable level of lymphocyte depletion was observed in the blood at the 0.1 mg/kg dose with CD4+ T cells and B cells being depleted to a greater extent than CD8+ T cells. These data also demonstrated that various T and B cell subsets are depleted to differing degrees depending on the dose of antibody used. Naïve T cells (both CD4 and CD8) demonstrated the most depletion compared to other cell populations (including memory and T reg cells), which were depleted to a lesser degree. In the B cell compartment, mature B cells were depleted more readily than immature B cells. In the spleen, dose dependent depletion was observed with significant depletion of lymphocytes being observed at the 5 and 1 mg/kg dose levels. Similar to Campath-1H®, naïve T cells were more readily depleted than memory cells. Depletion was observed for NK cells and neutrophils in the blood, but little to no depletion was observed in the spleen at any of the doses injected. Serum cytokine analysis demonstrated dose dependent increases for both TNF α and IL-6 with the 5 mg/kg dose inducing the highest level of each cytokine. Levels comparable to untreated mice were observed in the 0.5 and 0.1 mg/kg dose levels for TNF α and the 0.1 mg/kg dose level for IL-6. Dose dependent increases in the level of circulating MCP-1 were also noted.

[0486] By 30 days post dosing, lymphocyte levels for the 0.1 and 0.5 mg/kg groups had rebounded to the levels of untreated mice. In the 1.0 and 5.0 mg/kg groups, lymphocytes had

returned to normal levels by 80 days post dosing. Total lymphocytes were monitored for repopulation in the blood.

[0487] FIGS. 56A-56E show the level of CD4+ T cells, CD8+ T cells and B220 B cells in the blood 72 hours after dosing with 2C3-SFD1/K12 antibodies. FIGS. 57A-57E show the level of CD4+ T cells, CD8+ T cells and B220+ B cells in the spleen 72 hours after dosing with 2C3-SFD1/K12 antibodies. FIGS. 58A-58F show the levels of circulating cytokines 2 hours after dosing with 2C3-SFD1/K12 antibodies. FIG. 59 shows the repopulation of circulating lymphocytes over a timecourse after dosing with 2C3-SFD1/K12 antibodies.

Example 47: Analysis of Depletion and Repopulation of Anti-CD52 Antibodies in huCD52

10 Transgenic Mice (12G6-SFD1/K11)

[0488] The depleting activity of the 12G6-SFD1/K11 clone at different dose levels was examined in the huCD52 transgenic mouse. Mice were injected intravenously with 0.1, 0.5, 1.0 or 5.0 mg/kg of antibody. Two hours post dosing, serum was collected to potentially examine the level of circulating cytokines. Three days post dosing, mice were sacrificed, and blood and spleens were collected from each mouse (N=5) to determine the level of cell depletion using flow cytometry analysis. Samples were evaluated to determine the relative numbers of total T helper cell (CD4+), cytotoxic T cell (CD8+), B cell (B220+) and myeloid cell subpopulations present in the circulating peripheral blood or spleen of huCD52 transgenic mice. In addition, T and B cell subset analysis was performed to determine the overall depleting effect. A subset of mice (N=5) were kept alive to monitor the repopulation kinetics. Administration of 12G6-SFD1/K11 at the 5, 1, and 0.5 mg/kg doses resulted in depletion of a significant number of both T cells and B cells in the blood. A variable level of lymphocyte depletion was observed in the blood at the 0.1 mg/kg dose with CD4+ T cells and B cells being depleted to a greater extent than CD8+ T cells. These data also demonstrated that various T and B cell subsets are depleted to differing degrees depending on the dose of antibody used. Naïve T cells (both CD4 and CD8) demonstrated the most depletion compared to other cell populations (including memory and T reg cells), which were depleted to a lesser degree. In the B cell compartment, mature B cells were depleted more readily than immature B cells. In the spleen, dose dependent depletion was observed with significant depletion of lymphocytes being observed at the 5 and 1 mg/kg dose levels. Similar to Campath-1H®, naïve T cells were more readily depleted than memory cells. Depletion was observed for NK cells and neutrophils in the blood but little to no

depletion was observed in the spleen at any of the doses injected. Serum cytokine analysis demonstrated dose dependent increases for both TNF α and IL-6 with the 5 mg/kg dose inducing the highest level of each cytokine. Levels comparable to untreated mice were observed in the 0.5 and 0.1 mg/kg dose levels for TNF α and the 0.1 mg/kg dose level for IL-6. Dose dependent increases in the level of circulating MCP-1 were also noted.

[0489] By 30 days post dosing, lymphocyte levels had rebounded to the levels of untreated mice. In the 1.0 and 5.0 mg/kg groups, lymphocytes had returned to normal levels by 80 days post dosing. Total lymphocytes were monitored for repopulation in the blood.

[0490] FIGS. 60A-60E show the level of CD4+ T cells, CD8+ T cells and B220+ B cells in the blood 72 hours after dosing with 12G6-SFD1/K11 antibodies. FIGS. 61A-61E show the level of CD4+ T cells, CD8+ T cells and B220+ B cells in the spleen 72 hours after dosing with 12G6-SFD1/K11 antibodies. FIGS. 62A-62F show the levels of circulating cytokines 2 hours after dosing with 12G6-SFD1/K11 ("12G6 hu") antibodies. FIG. 63 shows the repopulation of circulating lymphocytes over a timecourse after dosing with 12G6-SFD1/K11 antibodies.

Example 48: Analysis of PK Profile of Anti-CD52 Antibodies in CD52 Transgenic Mice (2C3-SFD1/K12, 12G6-SFD1/K11 and 9D9-H10/K12)

[0491] The pharmacokinetic profiles of anti-CD52 antibodies were determined in huCD52 transgenic mice. This experiment compared the humanized and chimeric forms of the antibodies to ensure that the humanization process did not alter the clearance rate of the antibodies.

Comparisons included chimeric 2C3, 12G6, and 9D9 antibodies and humanized 2C3-SFD1/K12, 12G6-SFD1/K11, and 9D9-H10/K12 antibodies. Mice were injected i.v. with antibodies at 5 mg/kg and blood was collected at various timepoints beginning two hours post dosing. The circulating levels of each antibody were evaluated using an anti-human IgG ELISA. For each of the chimeric/humanized antibody pairs analyzed, there was a slight difference in the C_{max} noted at 2 hours post dosing. For the 2C3 and 12G6 antibodies, the C_{max} of the humanized version (i.e., 2C3-SFD1/K12 and 12G6-SFD1/K11) was slightly higher while the chimeric version was slightly higher for the 9D9 pair. Clearance rates for the antibody pairs were similar over the course of the experiment indicating that the humanization process did not significantly alter the pharmacokinetic profile of the antibodies.

[0492] FIGS. 64A-64C show the level of 2C3-chimeric, 2C3-SFD1/K12, 12G6-chimeric, 12G6-SFD1/K11, 9D9-chimeric, and 9D9-H10/K12 antibodies in the blood over a timecourse after dosing.

Example 49: Analysis of Depletion and Repopulation of Anti-CD52 Antibodies in huCD52

5 Transgenic Mice (9D9-H10/K12)

[0493] The depleting activity of the 9D9-H10/K12 clone at different dose levels was examined in the huCD52 transgenic mouse. Mice were injected intravenously with 0.1, 0.5, 1.0 or 5.0 mg/kg of antibody. Two hours post dosing, serum was collected to potentially examine the level of circulating cytokines. Three days post dosing, mice were sacrificed, and blood and spleens
10 were collected from each mouse (N=5) to determine the level of cell depletion using flow cytometry analysis. Samples were evaluated to determine the relative numbers of total T helper cell (CD4+), cytotoxic T cell (CD8+), B cell (B220+) and myeloid cell subpopulations present in the circulating peripheral blood or spleen of huCD52 transgenic mice. In addition, T and B cell subset analysis was performed to determine the overall depleting effect. A subset of mice
15 (N=5) were kept alive to monitor the repopulation kinetics. Administration of 9D9-H10/K12 at the 5, 1, and 0.5 mg/kg doses resulted in depletion of a significant number of both T cells and B cells in the blood. Only a modest level of lymphocyte depletion was observed in the blood at the 0.1 mg/kg dose. These data also demonstrated that various T and B cell subsets are depleted to differing degrees depending on the dose of antibody used. Naïve T cells (both CD4 and CD8)
20 demonstrated the most depletion compared to other cell populations (including memory and T reg cells), which were depleted to a lesser degree. In the B cell compartment, mature B cells were depleted more readily than immature B cells. In the spleen, significant depletion of these cells was only observed at the 5 and 1 mg/kg dose levels. Similar to Campath-1H®, naïve T cells were more readily depleted than memory cells. Depletion was observed for NK cells and
25 neutrophils in the blood but little to no depletion was observed in the spleen at any of the doses injected. Serum cytokine analysis demonstrated no significant increases for either TNF α or IL-6 at any of the dose levels analyzed. Dose dependent increases in the level of circulating MCP-1, however, were noted.

[0494] The repopulation portion of this experiment was terminated early when lymphocytes
30 were 50-80% repopulated (depending on the dose). Lymphocyte repopulation was monitored based on total lymphocyte counts and not on a T and B cell basis.

[0495] FIGS. 65A-65E show the level of CD4+ T cells, CD8+ T cells and B220+ B cells in the blood 72 hours after dosing with 9D9-H10/K12 ("9D9") antibodies. FIGS. 66A-66E show the level of CD4+ T cells, CD8+ T cells and B220+ B cells in the spleen 72 hours after dosing with 9D9-H10/K12 ("9D9") antibodies. FIGS. 67A-67F show the levels of circulating cytokines 2 hours after dosing with 9D9-H10/K12 ("9D9") antibodies. FIG. 68 shows the repopulation of circulating lymphocytes over a timecourse after dosing with 9D9-H10/K12 ("9D9") antibodies.

Example 50: Analysis of Depletion and Repopulation of Anti-CD52 Antibodies in huCD52 Transgenic Mice (2C3-SFD1/K12, 12G6-SFD1/K11 and 9D9-H10/K12)

[0496] The depleting activity of Campath-1H® and the humanized 2C3-SFD1/K12, 12G6-SFD1/K11 and 9D9-H10/K12 clones at different dose levels was examined in the huCD52 transgenic mouse. Mice were injected intravenously with either 0.1 or 1.0 mg/kg of antibody. Two hours post dosing, serum was collected to potentially examine the level of circulating cytokines. Three days post dosing, mice were sacrificed, and blood and spleens were collected from each mouse to determine the level of cell depletion using flow cytometry analysis. Samples were evaluated to determine the relative numbers of total T helper cell (CD4+), cytotoxic T cell (CD8+), B cell (B220+) and myeloid cell subpopulations present in the circulating peripheral blood or spleen of huCD52 transgenic mice. In addition, T and B cell subset analysis was performed to determine the overall depleting effect. All of the humanized antibodies (2C3-SFD1/K12, 12G6-SFD1/K11 and 9D9-H10/K12) mediated depletion of lymphocytes within the spleen and blood when compared with PBS treated animals. Depletion was more robust in the blood than the spleen for all antibodies, and the depletion was dose-dependent in both tissues. Depletion was most dramatic for CD4 and CD8+ T cells with less depletion in the B cell compartment. Various T and B cell subsets were depleted to differing degrees. Naïve T cells (both CD4 and CD8) demonstrated the most depletion compared to other cell populations (including memory and T reg cells), which were depleted to a lesser degree. In the B cell compartment, mature B cells were depleted more readily than immature B cells. Serum cytokine analysis revealed significant increases in the level of IL-6, MCP-1 and TNFα 2 hours post dosing. Increases were noted for all antibodies, including Campath-1H®, and were dose dependent (i.e. higher cytokine levels were noted for the 1.0 mg/kg dose level than the 0.1 mg/kg dose). In comparison to Campath-1H®, 2C3-SFD1/K12 and 12G6-SFD1/K11 induced similar levels of IL-6 while 9D9-H10/K12 induced IL-6 to a

significantly lower degree. For MCP-1, the 12G6-SFD1/K11 antibody induced lower levels, and both 12G6-SFD1/K11 and 9D9-H10/K12 decreased TNF α levels compared to Campath-1H®.

[0497] FIGS. 69A-69D show the level of bulk lymphocyte populations (CD4+ T cells, CD8+ T cells, and B cells) and CD4+ T cell, CD8+ T cell and B220+ B/NK cell subtypes in the blood 72 hours after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K12 ("2C3"), 12G6-SFD1/K11 ("12G6"), and 9D9-H10/K12 ("9D9") antibodies. FIGS. 70A-70D show the level of bulk lymphocyte populations (CD4+ T cells, CD8+ T cells, and B cells) and CD4+ T cell, CD8+ T cell and B220+ B/NK cell subtypes in the spleen 72 hours after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K12 ("2C3"), 12G6-SFD1/K11 ("12G6"), and 9D9-H10/K12 ("9D9") antibodies. FIGS. 71A-71F show the levels of circulating cytokines 2 hours after dosing with Campath-1H®, 2C3-SFD1/K12, 12G6-SFD1/K11, and 9D9-H10/K12 antibodies.

Example 51: Direct Comparison of Anti-huCD52 Humanized 9D9 Clones in huCD52

Transgenic Mice (9D9 H10/K12 and 9D9 H11/K12)

[0498] The depleting activity of two humanized anti-CD52 9D9 clones (9D9-H10/K12 and 9D9-H11/K12) was examined in huCD52 transgenic mice. Mice were injected intravenously with either 0.1 or 1.0 mg/kg of antibody. Three days post dosing, mice were sacrificed, and blood and spleens were collected from each mouse to determine the level of cell depletion using flow cytometry analysis. Samples were evaluated to determine the relative numbers of total T helper cell (CD4+), cytotoxic T cell (CD8+), B cell (B220+) and NK cell subpopulations present in the circulating peripheral blood or spleen of huCD52 transgenic mice. Treatment with either antibody resulted in similar lymphocyte depletion within the blood and spleen, with lymphocyte depletion in the blood being more robust. Further, CD4 and CD8+ T cells were more strongly depleted than B cells and NK cells in both tissues. While the depletion with the 9D9-H10/K12 clone appears less robust than the depletion with the 9D9-H11/K12 clone, the difference is not statistically significant.

[0499] FIG. 72 shows the level of CD4+ T cells, CD8+ T cells, B220+ B cells, and NK cells in the blood 72 hours after dosing with 9D9-H10/K12 and 9D9-H11/K12 antibodies. FIG. 73 shows the level of CD4+ T cells, CD8+ T cells, B220+ B cells, and NK cells in the spleen 72 hours after dosing with 9D9-H10/K12 and 9D9-H11/K12 antibodies.

Example 52: Direct Comparison of Anti-huCD52 Humanized 12G6 Clones in huCD52 Transgenic Mice (12G6-SFD1/K11 and 12G6-SFD1-K12)

[0500] The depleting activity of two humanized anti-CD52 12G6 clones (12G6-SFD1/K11 and 12G6-SFD1/K12) was examined in the huCD52 transgenic mouse. Mice were injected intravenously with either 0.1 or 1.0 mg/kg of antibody. Two hours post dosing, serum was collected to potentially examine the level of circulating cytokines. Three days post dosing, mice were sacrificed, and blood and spleens were collected from each mouse to determine the level of cell depletion using flow cytometry analysis. Samples were evaluated to determine the relative numbers of total T helper cell (CD4+), cytotoxic T cell (CD8+), B cell (B220+) and myeloid cell subpopulations present in the circulating peripheral blood or spleen of huCD52 transgenic mice. In addition, T and B cell subset analysis was performed to determine the overall depleting effect. Administration of either the 12G6-SFD1/K11 antibody or the 12G6-SFD1/K12 antibody resulted in a significant level of lymphocyte depletion within the blood. There appeared to be little to no difference in the lymphocyte depleting activity of the two clones. The pattern of lymphocyte depletion was such that naïve CD4 and CD8+ T cells were depleted to a higher degree than memory T cells or Treg cells. Myeloid cell populations were depleted to a lesser degree regardless of the clone (12G6-SFD1/K11 or 12G6-SFD1/K12) or dose. Serum cytokine analysis was not performed for this experiment.

[0501] FIGS. 74A-74D show the level of CD4+ T cells, CD8+ T cells, B220+ B/NK cells, and myeloid cells in the blood 72 hours after dosing with 12G6-SFD1/K11 ("12G6 K11") and 12G6-SFD1/K12 ("12G6 K12") antibodies. FIGS. 75A-75D show the level of CD4+ T cells, CD8+ T cells, B220+ B/NK cells, and myeloid cells in the spleen 72 hours after dosing with 12G6-SFD1/K11 ("12G6 K11") and 12G6-SFD1/K12 ("12G6 K12") antibodies.

Example 53: Direct Comparison of Anti-huCD52 Humanized 9D9 Clones in huCD52 Transgenic Mice (9D9 H11/K12, 9D9 H16/K13, and 9D9 H18/K13)

[0502] The depleting activity of three humanized 9D9 antibodies (9D9-H11/K12, 9D9-H16/K13, and 9D9-H18/K13) was compared in the huCD52 transgenic mouse. Human CD52 transgenic mice were treated with PBS as a vehicle control or injected with either 1mg/kg or 0.1mg/kg of each antibody. At two hours post dosing, serum was collected to determine the level of circulating cytokines. Three days later, mice were sacrificed, and peripheral blood and spleens were collected and processed for flow cytometry analysis. Samples were evaluated to

determine the relative numbers of total T helper cell (CD4+), cytotoxic T cell (CD8+), B cell (B220+) and myeloid cell subpopulations present in the circulating peripheral blood or spleen of huCD52 transgenic mice. In addition, T and B cell subset analysis was performed to determine the overall depleting effect. All 9D9 (9D9-H11/K12, 9D9-H16/K13, and 9D9-H18/K13) antibodies mediated cellular depletion of lymphocyte and myeloid cell populations in the blood and spleen to a similar extent. More robust lymphocyte and myeloid cell depletion was observed in the blood than the spleen. Comparison of the depleting activity of the 9D9 clones (9D9-H11/K12, 9D9-H16/K13, and 9D9-H18/K13) demonstrated that 9D9-H16/K13 resulted in the most robust depletion, followed by 9D9-H18/K13 and 9D9-H11/K12. This was most apparent for lymphocytes in the spleen at the 1mg/kg dose in which 9D9-H16/K13 treatment resulted in a higher degree of depletion than either of the other clones (9D9-H18/K13 and 9D9-H11/K12). Further, the pattern of depletion was such that naïve CD4 and CD8+ T cells were depleted to a higher degree than memory T cells or Treg cells, and B cell populations were depleted to a higher level with 9D9-H16/K13. Myeloid cell populations were less impacted by anti-CD52 treatment regardless of the clone of antibody (9D9-H11/K12, 9D9-H16/K13, or 9D9-H18/K13) or dose. Of the cytokines analyzed, increases were noted in IL-6, TNF α and MCP-1. Following injection, similar circulating level of IL6 and MCP-1 were observed for all of the 9D9 clones (9D9-H11/K12, 9D9-H16/K13, and 9D9-H18/K13) at both the 0.1 and 1.0 mg/kg dose levels. Slight differences were observed with circulating TNF α levels in which injection of the 9D9-H16/K13 clone resulted in a modest increase at the 1.0 mg/kg dose.

[0503] FIG. 76 shows the level of bulk lymphocyte populations (CD4+ T cells, CD8+ T cells, and B220+ B cells) in the blood 72 hours after dosing with 9D9-H11/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies. FIGS. 77A-77D show the level of CD4+ T cell, CD8+ T cell, B220+ B/NK cell, and myeloid cell subtypes in the blood 72 hours after dosing with 9D9-H11/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies. FIG. 78 shows the level of bulk lymphocyte populations (CD4+ T cells, CD8+ T cells, and B220+ B cells) in the spleen 72 hours after dosing with 9D9-H11/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies. FIGS. 79A-79D show the level of CD4+ T cell, CD8+ T cell, B220+ B/NK cell, and myeloid cell subtypes in the spleen 72 hours after dosing with 9D9-H11/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies. FIGS. 80A-80F show the levels of circulating cytokines 2 hours after dosing with 9D9-H11/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies.

Example 54: Analysis of PK Profile of Anti-CD52 Antibodies from the 2C3, 12G6, and 9D9 Families in CD52 Transgenic Mice

- [0504] The pharmacokinetic profiles of humanized 2C3-SFD1/K12, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13 and 9D9-H18/K13 were determined in huCD52 transgenic mice. Mice were injected i.v. with antibodies at 1mg/kg and blood was collected at various timepoints beginning two hours post dosing. The circulating levels of each antibody were evaluated using an anti-human Ig ELISA. The calculated half-lives were: 2C3-SFD1/K12 79.0 ± 23.9 hours, 12G6-SFD1/K11 49.0 ± 14.4 hours, 12G6-SFD1/K12 75.1 ± 28.5 , 9D9-H16/K13 59.8 ± 26.6 hours and 9D9-H18/K13 42.2 ± 15.7 hours.
- [0505] Overall, there was significant inter-animal variability for exposure in these studies. The terminal elimination half-lives for 2C3-SFD1/K12 and 12G6-SFD1/K12 were similar while the half-life of 12G6-SFD1/K11 was shorter but not significantly different. Clearance was fastest with 2C3-SFD1/K12 followed by 12G6-SFD1/K11 and 12G6-SFD1/K12. The two 12G6 treatments mirrored each other for most of the time points measured, while 2C3-SFD1/K12 showed less exposure and faster clearance. 9D9-H16/K13 and 9D9-H18/K13 were quite similar for all PK parameters measured.
- [0506] FIGS. 81A-81B show the level of 2C3-SFD1/K12, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13 and 9D9-H18/K13 antibodies in the blood over a timecourse after dosing.

TABLE 16

PK Parameters	2C3-SFD1/K12	12G6-SFD1/K11	12G6-SFD1/K12	9D9-H16/K13	9D9-H18/K13
$t_{1/2}$ (hr)	79.0 ± 23.9	49.0 ± 14.4	75.1 ± 28.5	59.8 ± 26.6	42.2 ± 15.7
Cl (ml/hr/kg)	20.3 ± 2.9	10.6 ± 1.69	7.08 ± 1.80	5.64 ± 1.73	6.65 ± 3.02
V_z (ml/kg)	2251 ± 539	770 ± 294	721 ± 224	445 ± 133	366 ± 100
AUC (ug*hr/ml)	251 ± 37.2	485 ± 104	747 ± 188	196 ± 70.2	174 ± 65.2
C_{max} (ug/ml)	4.22 ± 0.54	7.12 ± 1.97	8.96 ± 2.33	3.58 ± 2.16	4.35 ± 1.54

Example 55: Evaluation of Cytokine Storm in Response to Treatment with Anti-CD52 Antibodies

- [0507] The release of serum cytokine following treatment with anti-CD52 antibodies was evaluated in huCD52 transgenic mice. Animals were treated with 1 mg/kg of Campath-1H®, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13 or 9D9-H18/K13. One group of animals

was treated with 5mg/kg of 2C3-SFD1/K12 in view of previous results indicating that injection with 2C3-SFD1/K12 may result in lower levels of depletion compared to the other antibodies, thereby normalizing the groups based on the dose needed to achieve similar levels of depletion. All groups were bled 1, 2, 4, 24, and 48 hours post treatment and CBA analysis for inflammatory cytokines was conducted. All groups were also sacrificed 3 days post treatment and the spleens were evaluated for depletion of lymphocytes in the spleen by flow cytometry. Treatment with each of the antibodies resulted in depletion of various targets similar to that observed for Campath-1H®. This was also true for 2C3-SFD1/K12, in which a 5mg/kg dose was used to elicit similar depletion. Some variability in depletion was observed with 12G6-SFD1/K12 and 9D9-H16/K13, most likely due to the repeated bleeding of the animals to acquire serum for cytokine analysis. Cytokine expression, however, was reduced for antibodies from the 12G6 (12G6-SFD1/K11 and 12G6-SFD1/K12) and 9D9 (9D9-H16/K13 and 9D9-H18/K13) family members. This was most noticeable for release of IL-6, MCP-1 and TNF α at the early 1 and 2 hour time points.

[0508] FIGS. 82A-82F show the level of cytokines in the blood over a 48-hour timecourse following dosing with Campath-1H® ("Campath"), 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13 or 9D9-H18/K13 antibodies. FIGS. 83A-83E show the level of bulk lymphocytes, CD4+ T cells, CD8+ T cells, B220+ B/NK cells, and myeloid cells in the spleen 72 hours after dosing with Campath-1H® ("Campath"), 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13 or 9D9-H18/K13 antibodies.

Example 56: Evaluation of the Repopulation Kinetics in the Blood of CD52 Transgenic Mice Following Treatment with Anti-CD52 Antibodies

[0509] The repopulation kinetics of several cell types in the blood were assessed following administration of humanized anti-CD52 2C3-SFD1/K12, 9D9-H16/K13 and 12G6-SFD1/K12 antibodies. Mice were injected i.v. with each antibody at 2 mg/kg to ensure a robust level of depletion. At various timepoints post injection, blood was collected for flow cytometry analysis to determine the level of circulating lymphocytes in the blood, including CD4+ and CD8+ T cells, regulatory T cells, B cells, NK cells, neutrophils and macrophages. No differences were observed in the initial depleting activity for each antibody, which was confirmed on day 3 post injection. Mice were bled weekly for the first month and biweekly thereafter to monitor the kinetics of repopulation. The kinetics of lymphocyte repopulation were similar for any of the

anti-CD52 (2C3-SFD1/K12, 9D9-H16/K13 and 12G6-SFD1/K12) antibodies compared to Campath-1H®. By day 57, the B cells returned to baseline in the blood while T cells approached baseline levels by day 84. By day 116, CD8+ T cells had not returned to control levels, but similar repopulation kinetics for all other cell types monitored were observed with each of the anti-CD52 (2C3-SFD1/K12, 9D9-H16/K13 and 12G6-SFD1/K12) antibodies and Campath-1H®.

[0510] FIGS. 84A-84G show the repopulation of circulating CD4+ and CD8+ T cells, regulatory T cells, B cells, NK cells, neutrophils and macrophages over a timecourse after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K12, 9D9-H16/K13 and 12G6-SFD1/K12 antibodies.

Example 57: Evaluation of CD52 Expression in CD52 Transgenic Mice Using the Anti-CD52 Antibodies

[0511] Expression of huCD52 was evaluated using the humanized anti-CD52 antibodies to determine whether similar staining patterns could be observed on mature and developing cell populations in huCD52 transgenic mice. 2C3-SFD1/K12, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies were conjugated with FITC to use in flow cytometry staining. Tissues from huCD52 transgenic mice were collected and processed for staining. 2C3-SFD1/K12, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies stained lymphocytes expressing huCD52 from the spleen of transgenic mice similar to Campath-1H®. The staining patterns were representative of the lymphocyte populations and subsets found in other lymphoid organs such as the thymus and bone marrow.

[0512] FIG. 85 shows the ability of FITC-labeled Campath-1H®, 2C3-SFD1/K12, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies to specifically bind huCD52 lymphocyte cell populations in the spleen.

Example 58: Direct Comparison of Single Dose Treatment with Anti-huCD52 in huCD52 Transgenic Mice

[0513] The depleting activity of several humanized anti-CD52 antibodies (2C3-SFD1/K12, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13, and 9D9-H18/K13) was compared in the huCD52 transgenic mouse. Mice were injected with antibodies i.v. at 1 mg/kg. At 2-hours post dosing, serum was collected for cytokine analysis. Three days later mice were sacrificed and blood and spleen collected to compare the level of lymphocyte depletion. Significant levels of B

and T cell depletion were observed for all of the anti-CD52 antibodies (2C3-SFD1/K12, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13, and 9D9-H18/K13) and were comparable to those observed following Campath-1H® administration. Subset analysis also revealed no significant differences in the level of depletion for each antibody (2C3-SFD1/K12, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13, and 9D9-H18/K13) in either blood or spleen. Following injection of Campath-1H®, there was a marked increase in the circulating levels of both IL-6 and TNF α . Although injection of each of the anti-CD52 antibodies (2C3-SFD1/K12, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13, and 9D9-H18/K13) resulted in a significant decrease in the level of TNF α compared to Campath-1H®, the levels of IL-6 were similar.

[0514] FIGS. 86A-86E show the level of bulk lymphocyte populations (CD4+ T cells, CD8+ T cells, and B220+ B cells) and CD4+ T cell, CD8+ T cell, B220+ B/NK cell, and myeloid cell subtypes in the blood 72 hours after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K12, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies. FIGS. 87A-87E show the level of bulk lymphocyte populations (CD4+ T cells, CD8+ T cells, and B220+ B cells) and CD4+ T cell, CD8+ T cell, B220+ B/NK cell, and myeloid cell subtypes in the spleen 72 hours after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K12, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies. FIGS. 88A-88C show the levels of circulating cytokines 2 hours after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K12, 12G6-SFD1/K11, 12G6-SFD1/K12, 9D9-H16/K13, and 9D9-H18/K13 antibodies.

Example 59: In Depth Depletion of Lymphocytes in huCD52 Transgenic Mice Following Single Dose Treatment With Anti-huCD52 Antibodies

[0515] Extensive depletion analysis was performed in the huCD52 transgenic mouse using anti-CD52 2C3-SFD1/K12, 9D9-H16/K13 and 12G6-SFD1/K12 antibodies. Mice (N=4) were injected i.v. with a single dose of each antibody at 1 mg/kg. Three days later, the mice were sacrificed, and blood, spleen, lymph nodes, and thymus were collected to compare the level of lymphocyte depletion using multi-color flow cytometry analysis. Significant levels of B and T cell depletion were observed for all of the anti-CD52 2C3-SFD1/K12, 9D9-H16/K13 and 12G6-SFD1/K12 antibodies and were comparable to those observed following Campath1H® administration in each tissue examined. Subset analysis also revealed no significant differences

in the level of depletion for each antibody in either blood or spleen. Significant levels of lymphocyte depletion were also observed in the lymph nodes of mice. There did, however, appear to be some variability in the activity of the antibody, especially when looking at the central and effector memory T cell subset. Due to technical issues regarding the LSR-II and the

5 CD8 stain, the thymus could not be evaluated.

[0516] FIGS. 89A-89D show the level of CD4+ T cell, CD8+ T cell, B220+ B cell, and NK/myeloid cell subtypes in the blood 72 hours after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K12, 9D9-H16/K13 and 12G6-SFD1/K12 antibodies. FIGS. 90A-90D show the level of CD4+ T cell, CD8+ T cell, B220+ B cell, and NK/myeloid cell subtypes in the spleen
10 72 hours after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K12, 9D9-H16/K13 and 12G6-SFD1/K12 antibodies. FIGS. 91A-91D show the level of CD4+ T cell, CD8+ T cell, B220+ B cell, and NK/myeloid cell subtypes in the lymph node 72 hours after dosing with Campath-1H® ("Campath"), 2C3-SFD1/K12, 9D9-H16/K13 and 12G6-SFD1/K12 antibodies.

Example 60: Creation and Evaluation of the huCD52 Knock-in/Knock-out (KI/KO)

15 **Transgenic Mouse on the C57BL/6 Background**

[0517] A new human CD52 knock-in/knock-out mouse model was created on the C57BL/6 background. To create this mouse, the mouse CD52 gene sequence was replaced by the human CD52 gene sequence. The targeting strategy allowed for the replacement of the mouse sequence with the human sequence while maintaining the exon-intron structure. A selection marker was
20 used to identify progeny containing the new gene sequence. The final allele was created by removal of the selection marker leaving only the human CD52 gene sequence.

[0518] Basic characterization of the huCD52 KI/KO mouse model involved determining the level of human CD52 expression on lymphocytes. Blood from huCD52-KI/KO transgenic mice (N=4) and C57BL/6 mice (N=2) were stained for hCD52 expression and the number of CD52
25 molecules/cell was enumerated using the Bang's labs Simply Cellular anti-human antibody assay. Staining of peripheral blood cells from huCD52-KI/KO transgenic mice demonstrated that expression of huCD52 is very high on the majority of lymphocytes from these animals. Expression levels were similar to those observed in human CD4, CD8, and B cell populations. Expression levels on NK cells and macrophages were lower than those observed for T cells and
30 B cells. An increased level of huCD52 expression was detected on neutrophils in these mice, contrary to the decreased expression level in human neutrophils or similar cells from the original

transgenic mouse line on the CD-1 background. Similar levels of CD52 expression were observed on T and B cells from the original huCD52 CD1 transgenic mouse and the huCD52 KI/KI mouse.

[0519] FIG. 92A shows the huCD52 expression level on CD4+ T cell, CD8+ T cell, B220+ B cell, and NK/myeloid cell subtypes in huCD52-KI/KO and non-transgenic control mice. FIG. 92B shows the huCD52 expression level on CD4+ T cells, CD8+ T cells, and B cells in huCD52-KI/KO and huCD52 CD1 transgenic mice.

Example 61: Direct Comparison of Depletion Characteristics Between Small and Large Scale Lots of 12G6 and 2C3

[0520] huCD52 KI/KO transgenic mice were dosed with 12G6-SFD1/K12 or 2C3-SFD1/K12 to determine the depleting activity. In addition, activity was examined using antibodies generated from two different sources (small scale and large scale lots) at Genzyme. Mice were injected i.v. with each antibody at 1 mg/kg. Three days post injection, mice were sacrificed, and blood was collected for flow cytometry analysis to determine the levels of circulating CD4+ and CD8+ T cells, B cells, NK cells, neutrophils and macrophages. No significant differences in depletion of CD4 T cells, CD8+ T cells, B cells, and NK cells were observed between the small scale and large scale lot derived antibodies.

[0521] The various lots of 12G6-SFD1/K12 and 2C3-SFD1/K12 antibodies were also evaluated by flow cytometry to compare the intensity of staining on splenocytes from huCD52-KI/KO transgenic mice. Both 12G6-SFD1/K12 and 2C3-SFD1/K12 antibodies appear to recognize human CD52 to the same extent as Campath-1H® on isolated splenocytes. In addition, there was no difference in the level of recognition between the two sources (small scale and large scale lots) of antibody.

[0522] FIGS. 93A-93B show binding to huCD52 of 12G6-SFD1/K12 and 2C3-SFD1/K12 antibodies (from various production sources) as compared to a Campath-1H® control. FIG. 94 shows the level of bulk lymphocyte populations (CD4+ T cells, CD8+ T cells, and B220+ B cells) in the blood 72 hours after dosing with 12G6-SFD1/K12 and 2C3-SFD1/K12 antibodies from various production sources.

Example 62: Analysis of PK Profile for Anti-CD52 Antibodies in huCD52-KI/KO Transgenic Mice

[0523] The pharmacokinetic profiles of humanized 2C3-SFD1/K12, 9D9-H16/K13 and 12G6-SFD1/K12 antibodies were determined in huCD52 KI/KO transgenic mice. Mice were injected i.v. with antibodies at 1mg/kg, and blood was collected at various timepoints beginning two hours post dosing. The circulating levels of each antibody were evaluated using an anti-human Ig ELISA. The overall clearance rate was similar for each of the humanized anti-CD52 2C3-SFD1/K12, 9D9-H16/K13 and 12G6-SFD1/K12 antibodies with 2C3-SFD1/K12 exhibiting potentially faster kinetics, while 12G6-SFD1/K12 was present in the serum for the longest period of time.

[0524] FIGS. 95A-95B show the levels of 2C3-SFD1/K12, 9D9-H16/K13 and 12G6-SFD1/K12 antibodies in the blood over a timecourse after dosing.

Example 63: Evaluation of 12G6 and 2C3 Pretreatment on EAE in huCD52-KI/KO Transgenic Mice

[0525] The efficacy of anti-CD52 antibody treatment on reducing the overall disease incidence and severity of Experimental Autoimmune Encephalomyelitis (EAE) was evaluated in huCD52 KI/KO mice. huCD52-KI/KO mice were treated with a course of either 2C3-SFD1/K12 or 12G6-SFD1/K12 on days -5 thru -1. EAE (a model of multiple sclerosis) was induced by immunization with MOG35-55 peptide emulsified in CFA, and treatment with pertussis toxin, on days 0 and 2. Vehicle treated mice began to display signs of paralysis by day 10 post injection, which developed into severe progressive disease. In contrast, pretreatment of mice with either the 2C3-SFD1/K12 or 12G6-SFD1/K12 antibody delayed the onset of disease and decreased the overall disease severity.

[0526] FIG. 96 demonstrates the EAE clinical score of 2C3-SFD1/K12 and 12G6-SFD1/K12 over a timecourse of disease progression.

Example 64: Fc Modification of Antibodies to Alter the Pharmacokinetic Profile of Anti-CD52 Antibodies

[0527] Alterations in the Fc region of antibodies 1) affect the biological activity of the antibody by altering interactions with Fc receptors and/or 2) alter the pharmacokinetic profile of the antibody by altering interactions with the FcRn neonatal receptor. The FcRn molecule is expressed on vascular endothelium and is believed to be the main site of IgG recycling. The

FcRn binds to the antibody Fc portion which then becomes internalized within a cell.

Antibodies that have high affinity interactions with the FcRn will be recycled back to the surface of the cell and will be released back into circulation. Antibodies that have lower affinity interactions dissociate within the cell and ultimately degrade. Site directed mutagenesis to

- 5 increase the interaction with FcRn generates an antibody that can be maintained in circulation for longer periods of time compared to an unmodified antibody. Conversely, mutations within the Fc region of an antibody that decrease FcRn binding shorten the circulating half-life of the antibody. Mutations that have been described to decrease binding to FcRn resulting in shorter circulating half-lives include the His435Ala single mutation and the His310Ala/His435Gln
- 10 double mutation (see, e.g., Kim et al., "Mapping the site on human IgG for binding of the MHC class I-related receptor, FcRn," *Eur. J. Immunol.*, 29:2819-2825 (1999) and Kenanova et al., "Tailoring the Pharmacokinetics and Positron Emission Tomography Imaging Properties of Anti-Carcinoembryonic Antigen Single-Chain Fv-Fc Antibody Fragments," *Cancer. Res.* 65(2):622-631 (2005)).

- 15 **[0528]** The 2C3-SFD1/K12 antibody was mutated to generate His435Ala 2C3-SFD1/K12 ("2C3-SFD1/K12-Modified 1") and His310Ala/His435Gln 2C3-SFD1/K12 ("2C3-SFD1/K12-Modified 2") antibodies that have altered PK profiles. Biacore analysis was conducted to confirm decreased binding to both mouse and human FcRn molecules. Both Campath-1H® and 2C3-SFD1/K12 antibodies bound to each of the mouse and human FcRn molecules with similar
- 20 kinetics. In contrast, His435Ala 2C3-SFD1/K12 antibodies bound at low levels to the mouse FcRn but not to human FcRn. His310Ala/His435Gln 2C3-SFD1/K12 antibodies did not bind to either mouse or human FcRn molecule, indicating that the incorporation of either the single or double mutation into the 2C3-SFD1/K12 Fc region significantly affects binding to mouse and human FcRn.

- 25 **[0529]** FIGS. 97A-97B demonstrate the ability of Campath1H® ("Campath"), 2C3-SFD1/K12 ("2C3"), His435Ala 2C3-SFD1/K12 ("H435A 2C3") and His310Ala/His435Gln 2C3-SFD1/K12 ("H310A/H435Q 2C3") to bind to mouse and human FcRn molecules.

Example 65: Evaluation of the Half-Life of Fc Modified Anti-CD52 Antibodies Following I.V. Administration in C57Bl/6 Mice

- 30 **[0530]** Fc modifications were incorporated into the 2C3-SFD1/K12 backbone to generate 2C3-SFD1/K12-Modified 1 and 2C3-SFD1/K12-Modified 2 antibodies that exhibited decreased

binding to the FcRn receptor responsible for maintaining antibodies in circulation. The pharmacokinetic profile was determined for the 2C3-SFD1/K12 antibody and the 2C3-SFD1/K12-Modified 1 and 2C3-SFD1/K12-Modified 2 antibodies with reduced FcRn binding. C57BL/6 mice were used to evaluate the PK profile in the absence of target antigen (2C3-SFD1/K12 binds to human CD52 but does not cross-react with mouse CD52). Mice were injected i.v. with antibodies at 1 mg/kg. At various timepoints, blood was collected to analyze the level of circulating human IgG1 in the mouse serum by ELISA. Both 2C3-SFD1/K12-Modified 1 and 2C3-SFD1/K12-Modified 2 antibodies were cleared from the blood faster than the 2C3-SFD1/K12 antibody. 2C3-SFD1/K12 had a half-life of 403 hrs, while 2C3-SFD1/K12-Modified 1 had a half-life of 51 hours and 2C3-SFD1/K12-Modified 2 had a half-life of 8 hours. PK profiles for 2C3-SFD1/K12 and 2C3-SFD1/K12-Modified-1 were consistent with a 1-compartment model with only a single phase of elimination. In contrast, profiles for 2C3-SFD1/K12-Modified-2 were consistent with a 2 compartment model, with 2 distinct phases of elimination (specified as alpha and beta in the table). The first phase lasted until 48 hr post dose (alpha) and the second phase (beta, also called the terminal elimination phase) started 48hr post dose.

[0531] FIG. 98 shows the in vivo clearance of 2C3-SFD1/K12 ("2C3 unmodified"), 2C3-SFD1/K12-Modified 1 ("2C3-Fc mutant 1") and 2C3-SFD1/K12-Modified 2 ("2C3-Fc mutant 2") in nontransgenic mice.

Table 17-Summary of Pharmacokinetic Data Across Groups

	2C3-SFD1/K12	2C3-SFD1/K12-Modified 1	2C3-SFD1/K12-Modified 2
$t_{1/2}$ (hr)	403 $\pm$ 140	51.0 $\pm$ 12.3	8.05 $\pm$ 0.74 (Alpha) 282 $\pm$ 385 (Beta)
Cl (ml/hr/kg)	0.29 $\pm$ 0.09	1.35 $\pm$ 0.36	5.90 $\pm$ 4.67
V _z (ml/kg)	156 $\pm$ 40.7	94.8 $\pm$ 14.3	1932 $\pm$ 1341
AUC (ug*hr/ml)	3748 $\pm$ 937	781 $\pm$ 171	230 $\pm$ 105
C _{max} (ug/ml)	9.65 $\pm$ 1.72	11.9 $\pm$ 0.83	9.64 $\pm$ 3.70

Table 18—Individual Animal Data

Group [#]	Animal	HL_Lambda_z (hr)	C _{max} (ug/ml)	AUCINF_obs (hr*ug/ml)	V _{z_obs} (ml/kg)	Cl_obs (ml/hr/kg)
2C3	2.1	197.26	11.56	2967.86	95.89	0.34
2C3	2.2	494.01	10.54	4635.96	153.73	0.22
2C3	2.3	324.61	10.06	3783.76	123.77	0.26
2C3	2.4	283.68	10.57	3130.92	130.72	0.32
2C3	2.5	330.89	6.15	2025.29	235.71	0.49
2C3	2.6	547.78	10.56	4469.73	176.81	0.22
2C3	2.7	597.92	10.57	4764.75	181.04	0.21

2C3	2.8	320.65	7.61	3415.82	135.43	0.29
2C3	2.9	527.01	9.27	4533.82	167.70	0.22
	AVG	402.65	9.65	3747.55	155.64	0.29
	SD	140.22	1.72	937.38	40.73	0.09

Group [#]	Animal	HL_Lambda_z (hr)	Cmax (ug/ml)	AUCINF_obs (hr*ug/ml)	Vz_obs (ml/kg)	Cl_obs (ml/hr/kg)
2C3-M1	3.1	35.20	12.84	513.50	98.89	1.95
2C3-M1	3.2	42.74	11.68	842.55	73.17	1.19
2C3-M1	3.3	50.55	11.39	902.62	80.80	1.11
2C3-M1	3.4	46.61	12.49	717.95	93.67	1.39
2C3-M1	3.5	56.38	12.94	911.32	89.26	1.10
2C3-M1	3.6	63.40	12.41	995.22	91.91	1.00
2C3-M1	3.7	33.86	12.02	513.17	95.19	1.95
2C3-M1	3.8	63.14	10.56	842.79	108.08	1.19
2C3-M1	3.9	66.75	11.02	788.59	122.12	1.27
	AVG	50.96	11.93	780.86	94.79	1.35
	SD	12.30	0.83	170.51	14.33	0.36

Group [#]	Animal	Alpha HL_Lambda (hr)	Beta HL_Lambda (hr)	Cmax (ug/ml)	AUCINF_obs (hr*ug/ml)	Vz_obs (ml/kg)	Cl_obs (ml/hr/kg)
2C3-M2	4.1*	8.31	Missing	10.62	177.07	67.74	5.65
2C3-M2	4.2	7.42	994.71	11.35	390.03	3679.37	2.56
2C3-M2	4.3	7.37	703.09	10.48	315.82	3211.80	3.17
2C3-M2	4.4	7.72	227.03	11.78	247.96	1320.92	4.03
2C3-M2	4.5**	Missing	Missing	Missing	Missing	Missing	Missing
2C3-M2	4.6**	Missing	Missing	Missing	Missing	Missing	Missing
2C3-M2	4.7***	77.89	77.89	1.32	61.71	1820.87	16.20
2C3-M2	4.8	8.18	150.98	11.41	221.89	981.64	4.51
2C3-M2	4.9	9.33	77.61	10.49	194.31	576.21	5.15
	AVG	8.05	281.98	9.64	229.83	1931.80	5.90
	SD	0.74	384.82	3.70	104.69	1341.43	4.67

#-The tested groups were 2C3-SFD1/K12 ("2C3"), 2C3-SFD1/K12-Modified 1 ("2C3-M1") and 2C3-SFD1/K12-Modified 2 ("2C3-M2")

*Animal 4.1 no beta t_{1/2}, Vz outlier.

** - Animals 4.5 & 4.6, not enough data for PK analysis.

***Animal 4.7 incomplete injection

Example 66: Evaluation of the Half-Life of Fc Modified Anti-CD52 Antibodies Following

I.V. Administration in Heterozygous huCD52 Transgenic Mice

- 5 [0532] The pharmacokinetic profile was determined for the 2C3-SFD1/K12 antibody and the 2C3-SFD1/K12-Modified 1 and 2C3-SFD1/K12-Modified 2 antibodies with reduced FcRn binding in vitro. huCD52 transgenic mice were used to evaluate the PK profile in the presence

of the 2C3-SFD1/K12 antibody target antigen. Mice were injected i.v. with antibodies at 1 mg/kg. At various timepoints, blood was collected to determine the level of circulating human IgG1 in the mouse serum by ELISA. Both 2C3-SFD1/K12-Modified 1 and 2C3-SFD1/K12-Modified 2 antibodies were cleared from the blood faster than the 2C3-SFD1/K12 antibody.

- 5 2C3-SFD1/K12 had a half-life of 64 hrs, while 2C3-SFD1/K12-Modified 1 had a half-life of 32 hours, and 2C3-SFD1/K12-Modified 2 had a half-life of 6.5 hours.

[0533] FIG. 99 shows the in vivo clearance of 2C3-SFD1/K12 ("2C3"), 2C3-SFD1/K12 modified 1 ("2C3-Fc mutant 1") and 2C3-SFD1/K12 modified 2 ("2C3-Fc mutant 2") in huCD52 transgenic mice.

10 Table 19—Summary of Pharmacokinetic Data Across Groups

	2C3-SFD1/K12	2C3-SFD1/K12-Modified 1	2C3-SFD1/K12-Modified 2
$t_{1/2}$ (hr)	64.2 ± 12.1	32.3 ± 3.25	6.58 ± 2.03
Cl (ml/hr/kg)	2.15 ± 0.31	2.51 ± 0.28	5.41 ± 0.83
Vz (ml/kg)	198 ± 42.8	117 ± 21.1	49.7 ± 11.1
AUC (ug*hr/ml)	475 ± 73.4	403 ± 44.5	188 ± 27.2
Cmax (ug/ml)	8.88 ± 1.69	12.4 ± 1.67	12.9 ± 1.91

Table 20—Individual Animal Data

Group#	Animal	HL_Lambda_z (hr)	Cmax (ug/ml)	AUCINF_obs (hr*ug/ml)	Vz_obs (ml/kg)	Cl_obs (ml/hr/kg)
2C3	2.1	77.32	8.19	421.87	264.42	2.37
2C3	2.11	61.47	10.38	483.25	183.51	2.07
2C3	2.2	78.28	9.28	496.58	227.42	2.01
2C3	2.3	82.38	6.99	441.98	268.91	2.26
2C3	2.4	53.60	9.08	465.09	166.28	2.15
2C3	2.5	58.02	9.09	526.59	158.95	1.90
2C3	2.6	44.97	6.03	371.17	174.78	2.69
2C3	2.7	56.52	9.38	476.41	171.16	2.10
2C3	2.8	67.99	12.13	641.28	152.97	1.56
2C3	2.9	61.46	8.30	421.65	210.29	2.37
	Mean	64.20	8.88	474.59	197.87	2.15
	SD	12.06	1.69	73.40	42.80	0.31

Group#	Animal	HL_Lambda_z (hr)	Cmax (ug/ml)	AUCINF_obs (hr*ug/ml)	Vz_obs (ml/kg)	Cl_obs (ml/hr/kg)
2C3-M1	3.1	28.48	15.19	412.41	99.64	2.42
2C3-M1	3.11	34.64	12.60	468.36	106.69	2.14
2C3-M1	3.2	27.57	14.17	411.82	96.60	2.43
2C3-M1	3.3	34.27	11.96	401.38	123.20	2.49
2C3-M1	3.4	29.10	12.51	400.36	104.85	2.50
2C3-M1	3.5	29.63	11.11	470.98	90.77	2.12

2C3-M1	3.6	32.76	9.75	348.72	135.53	2.87
2C3-M1	3.7	35.68	10.41	328.71	156.61	3.04
2C3-M1	3.8	36.41	13.52	390.24	134.61	2.56
2C3-M1	3.9	34.06	12.39	392.53	125.19	2.55
Mean		32.26	12.36	402.55	117.37	2.51
SD		3.25	1.67	44.48	21.05	0.28

Group#	Animal	HL_Lambda_z (hr)	Cmax (ug/ml)	AUCINF_obs (hr*ug/ml)	Vz_obs (ml/kg)	Cl_obs (ml/hr/kg)
2C3-M2	4.1	7.64	13.45	197.24	55.85	5.07
2C3-M2	4.11	Missing	9.00	Missing	Missing	Missing
2C3-M2	4.2	7.79	14.92	217.80	51.61	4.59
2C3-M2	4.3	7.35	12.79	183.44	57.78	5.45
2C3-M2	4.4	3.54	10.34	152.92	33.44	6.54
2C3-M2	4.5	Missing	Missing	Missing	Missing	Missing
2C3-M2	4.6	Missing	Missing	Missing	Missing	Missing
2C3-M2	4.7	Missing	Missing	Missing	Missing	Missing
2C3-M2	4.8	Missing	Missing	Missing	Missing	Missing
2C3-M2	4.9	Missing	Missing	Missing	Missing	Missing
Mean		6.58	12.10	187.85	49.67	5.41
SD		2.03	2.40	27.23	11.12	0.83

PK parameters not available for 4.11, 4.5, 4.6, 4.7, 4.8, and 4.9 due to insufficient data.

[0534] #The tested groups were 2C3-SFD1/K12 ("2C3"), 2C3-SFD1/K12-Modified 1 ("2C3-M1") and 2C3-SFD1/K12-Modified 2 ("2C3-M2")

Example 67: Evaluation of In Vivo Depletion Following I.V. Administration of Fc

5 Modified Anti-CD52 Antibodies in Heterozygous huCD52 Transgenic Mice

[0535] The depletion activity was determined for the 2C3-SFD1/K12, 2C3-SFD1/K12-Modified 1, and 2C3-SFD1/K12-Modified 2 antibodies in huCD52 transgenic mice. Mice were treated with 1 mg/kg of 2C3-SFD1/K12, 2C3-SFD1/K12-Modified 1, or 2C3-SFD1/K12-Modified 2 antibodies and evaluated for the presence of CD4⁺ T cells, CD8⁺ T cells, B cells, and NK cells 72 hours later. Administration of 2C3-SFD1/K12-Modified 1 or 2C3-SFD1/K12-Modified 2 antibodies resulted in decreased levels of depletion in the blood and spleen compared administration of 2C3-SFD1/K12 antibodies. Further, 2C3-SFD1/K12-Modified 1 elicited greater depletion than 2C3-SFD1/K12-Modified 2 in both the blood and spleen

[0536] FIGS. 100A-100B show the level of bulk lymphocyte populations (CD4⁺ T cells, CD8⁺ T cells, B220⁺ B cells, and NK cells) in the blood and spleen 72 hours after dosing with 2C3-SFD1/K12 ("2C3"), 2C3-SFD1/K12 modified 1 ("2C3 Fc mutant-1"), and 2C3-SFD1/K12 modified 2 ("2C3 Fc mutant-2") antibodies.

Example 68: Detailed Epitope Specificities of Humanized Anti-CD52 Antibodies

[0537] Detailed epitope specificities of the humanized 12G6-SFD1/K12, 2C3-SFD1/K12, and 9D9-H16/K13 antibodies were determined using a Biacore T100 instrument. As a control, the epitope specificity of clone 097 (purified anti-human CD52 antibody, Biolegend) was evaluated using the same methodologies. The epitope specificity of clone 097 had previously been characterized using a peptide ELISA method (Hale G, "Synthetic peptide mimotype of the CAMPATH-1 (CD52) antigen, a small glycosylphosphatidylinositol-anchored glycoprotein," *Immunotechnology*, 1:175-187 (1995)). In this Biacore T100 assay, the antibodies were directly immobilized on Biacore CM5 Series S carboxymethyl dextran sensor chips (GE #BR-1006-68) using amine coupling. The carboxymethyl dextran surface was activated using a 1:1 mixture of 0.1M N-hydroxysuccinimide (NHS) and 0.4M N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), allowing the surface to bind reactive amine groups on the antibodies. Because IgM antibodies tend to have a higher level of non-specific binding compared to IgGs, the binding of a mouse IgM κ (mIgM κ) isotype control (Biolegend clone #MM-30) was also investigated. Following antibody immobilization, the reactive sensor chip surface was quenched using 1M ethanolamine hydrochloride/NaOH pH 8.5. One flow cell on each chip was a blank reference surface, and subsequent flow cells were immobilized with 10,000 RU of antibody.

[0538] A series of alanine-scanning mutant peptides comprising the human CD52 sequence (MUT 1 – MUT 12 (SEQ ID NOS: 169-180, respectively), Table 21) (see, e.g., Hale G, "Synthetic peptide mimotype of the CAMPATH-1 (CD52) antigen, a small glycosylphosphatidylinositol-anchored glycoprotein," *Immunotechnology*, 1:175-187 (1995)) were synthesized. Antibody binding to these mutant CD52 peptides and to wildtype human CD52 peptides was tested at concentrations of 500 nM, 100 nM, 50 nM, and 0 nM. Peptides were diluted into the assay running buffer, HBS-EP+ (10 mM HEPES, 150 mM NaCl, 0.05% P20 surfactant, 3 mM EDTA, pH 7.4). Duplicates of 100nM samples were included. The light (κ) chain specific rat anti-mouse IgM antibody (Southern Biotech Clone #1B4B1) was also included as an IgM control. The T100 instrument sample chamber and assay temperatures were set to 4°C and 25°C, respectively. The human CD52 peptide samples were injected for five minutes at a 50 μ l/min flow rate to measure association, and washed in HBS-EP+ for five minutes at a 50 μ l/min flow rate to measure dissociation. The antibody surface was stripped of

any remaining bound peptide using a sixty second injection of 10 mM glycine-HCl pH 2.0 at a 50 µl/min flow rate. Analysis was performed using Biacore T100 Kinetics Evaluation software v2.0 (GE Healthcare). Data was fit to a 1:1 model with reference flow cell and 0 nM concentration subtraction (double-reference subtraction). Representative sensorgrams of 12G6-SFD1/K12 antibody negative ((-), MUT 8) and positive ((+), MUT 9) peptide epitope recognition are shown in FIG. 101A and FIG. 101B, respectively. The compiled peptide binding data is summarized in Table 21.

[0539] The previously characterized binding specificity of clone 097 (Hale G, "Synthetic peptide mimotype of the CAMPATH-1 (CD52) antigen, a small glycosylphosphatidylinositol-anchored glycoprotein," *Immunotechnology*, 1:175-187 (1995)) was determined by coating ELISA plates with peptides containing the six residues of the C-terminal portion of human CD52 and then measuring the binding of the antibody to the fixed peptide. Each of the residues was substituted by all 20 amino acids. Because the peptides were attached to a solid surface in this ELISA, the assay may have been more influenced by avidity effects than the Biacore T100 assay described herein, which uses an antibody fixed to the surface over which the peptides are flowed. In the ELISA study, alanine substitutions at positions 11 and 12 (wildtype residues proline and serine, respectively) of the mature form of human CD52 were found to reduce strong binding of clone 097 to the peptide. In the present Biacore T100 study, alanine substitutions at positions 11 and 12 (as well as positions 7, 8, 9, and 10) were found to abrogate binding of clone 097. The hypothesized avidity effects of the ELISA assay are likely the reason why the mapped epitope of clone 097 is smaller as determined by the ELISA method than as determined by the described Biacore T100 assay.

[0540] The binding of both the 2C3-SFD1/K12 and 12G6-SFD1/K12 humanized antibodies to the human CD52 peptide sequence is sensitive to alanine substitutions at positions 7, 8, and 11 and the binding of humanized 9D9-H16/K13 is sensitive to alanine substitutions at positions 4 and 11. These defined epitope specificities overlap with the results observed in Example 4 (summarized in Table 8). Slight variations between the results are not unexpected given that the Biacore T100 method used to measure binding in the present case was significantly different from the method used in Example 4. In contrast to the present case, in Example 4, engineered CHO cells were used to express wildtype or alanine-substituted mutants of human CD52. Human CD52 expressed in such mammalian cells can be glycosylated, affecting binding. This is not the case for the human CD52 used in the Biacore T100 assay.

Table 21: Binding to alanine-scanning mutant hCD52 peptides

Peptide	Peptide Sequence	2C3-SFD1/K12 Binding	9D9-H16/K13 Binding	12G6-SFD1/K12 Binding	097 Binding	Control mIgM Binding
MUT 1	AQN ~~A~~ DTSTSSPSADC	+	+	+	+	-
MUT 2	G ~~A~~ NDTSQTSSPSADC	+	+	+	+	-
MUT 3	GQ ~~A~~ DTSTSSPSADC	+	+	+	+	-
MUT 4	GQN ~~A~~ TSTSSPSADC	+	-	+	+	-
MUT 5	GQND ~~A~~ SQTSSPSADC	+	+	+	+	-
MUT 6	GQNDT ~~A~~ QTSSPSADC	+	+	+	+	-
MUT 7	GQNDTS ~~A~~ TSSPSADC	-	+	-	-	-
MUT 8	GQNDTSQ ~~A~~ SSPSADC	-	+	-	-	-
MUT 9	GQNDTSQT ~~A~~ SPSADC	+	+	+	-	-
MUT 10	GQNDTSQTS ~~A~~ PSADC	+	+	+	-	-
MUT 11	GQNDTSQTSS ~~A~~ SADC	-	-	-	-	-
MUT 12	GQNDTSQTSSP ~~A~~ ADC	+	+	+	-	-
Controls						
WT 1	GQNDTSQTSSPSADK-Biotin	+	+	+	+	-
WT 2	Biotin-GQNDTSQTSSPSAD	+	-	+	+	-
Rat anti-mIgM	N/A	N/A	N/A	N/A	+	+

(+) Binding detected: Maximum response ($R_{\max}$) > 2RUs for 500nM peptide injection

(-) No binding detected: Maximum response ($R_{\max}$) < 2RUs for 500nM peptide injection

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Example 69: Assessment of CD4+ T cell responses induced by Campath-1H® or 12G6-SFD1/K12

[0541] The CD4+ T cell proliferative response was evaluated after repeated *in vitro* stimulation with autologous dendritic cells (DC) preloaded with a set of overlapping 15-mer peptides comprising sequences from the variable regions of either Campath-1H® or the humanized 12G6-SFD1/K12 antibody. These experiments utilized normal human donor T cells and DCs. Results were measured by quantifying tritiated thymidine incorporation of the proliferating human CD4+ T cells in response to autologous peptide pulsed antigen presenting cells (APC).

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15 [0542] **Cell preparation:** PBMCs were isolated from a normal human donor apheresis product acquired from BioMed Supplies (Carlsbad, CA). HLA haplotype screening of the donor blood was performed by Key Biologics, LLC (Memphis, TN) (Table 22). PBMCs were isolated

using the Ficoll-Paque PLUS density gradient (GE Healthcare) and a series of washes with phosphate buffered saline (PBS, Invitrogen, Carlsbad, CA). CD4+ T cells were isolated from PBMC using the Dynal CD4+ bead-based positive isolation kit (Invitrogen), following the manufacturer's recommended protocol. Isolated CD4+ T cells were frozen in Recovery Cell Culture Freezing Media (Invitrogen) and stored in liquid nitrogen. Dendritic Cells (DC) were induced from PBMCs by plating adherent cells with GM-CSF (Leukine, Bayer, Leverkusen, Germany) and IL-4 (Peprotech, Rocky Hill, NJ) for six days. Media supplemented with GM-CSF and IL-4 was replaced on day 4. DCs were subsequently isolated from the flasks and frozen in the Freezing Media then transferred to liquid nitrogen storage tanks.

Table 22: HLA haplotype of blood donors

Donor	HLA DR haplotype		peptide set
BMS170	DRB1 0701	DRB1 1503	Campath
BMS154	DRB1 0301	DRB1 0302	Campath
BMS150	DRB1 1101	DRB1 1302	Campath
BMS167	DRB1 0701	DRB1 1503	Campath
BMS200	DRB1 0804	DRB1 1202	Campath
BMS301	DRB1 1401	DRB1 1503	Campath
BMS352	DRB1 0301	DRB1 1101	Campath
BMS362	DRB1 0302	DRB1 0302	Campath
BMS484	DRB1 0103	DRB1 1201	Campath/GLD52
BMS486	DRB1 1302	DRB1 1303	Campath/GLD52
BMS640	DRB1 0301	DRB1 1302	GLD52
BMS656	DRB1 301	DRB1 1101	GLD52
BMS902	DRB1 0302	DRB1 0804	GLD52
BMS928	DRB1 1001	DRB1 1503	GLD52
BMS927	DRB1 1001	DRB1 1503	GLD52
BMS963	DRB1 0302	DRB1 1401	GLD52
BMS361	DRB1 1102	DRB1 1401	GLD52
BMS165	DRB1 1102	DRB1 1501	GLD52

[0543] Peptide: Peptides encompassing the heavy and light chain variable regions of Campath-1H® and 12G6-SFD1/K12 were synthesized using a Rainin Symphony automated peptide synthesizer using standard Fmoc-chemistry on CLEAR resin (Peptides International, Louisville, KY). Amino acids (EMD Biosciences, San Diego, CA or Anaspec, San Jose, Ca) were orthogonally protected with tert-Butoxycarbonyl (BOC), tert-Butyl (tBu), 2,2,4,6,7-Pentamethyldihydro-benzofuran-5-sulfonyl (Pbf), or Trityl (Trt) groups. Couplings were performed using an amino acid/HCTU/HOBt/DIEA/resin with a molar ratio of 6:6:3:12:1. A solution of 20% Piperidine and 2.5% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in DMF was

used to remove Fmoc from the amino terminus during each cycle. Deprotection/cleavage from resin was performed using a cocktail of 15mls/0.1mM resin of 2.5% water/2.5% TIS/5% Anisole/90% TFA v/v ratio for 3 hours. Supernatant was precipitated in diethyl-ether (-80°C) and pelleted at 3000 rpm for 10 minutes. Ether was decanted and the pellet was washed again. Crude peptide was then lyophilized. Analytical HPLC (XBridge C18 4.5 x 100mm, Waters Corp., Milford, MA) and MALDI-TOF mass spectrometry (Synapt, Waters Corp., Milford, MA) were used to verify the sequences and assess purity. All reagents were HPLC grade (EMD Biosciences, San Diego, Ca or Sigma Aldrich, St. Louis, MO). Lyophilized peptides were resuspended in 100% DMSO (Sigma). Forty three Campath-1H® peptides were combined into 11 linear groups, each containing 3 or 4 peptides per group (Table 23: from top to bottom, light chain peptides are denoted by SEQ ID NOs: 187-206 and heavy chain peptides are denoted by SEQ ID NOs: 207-229). The 42 12G6-SFD1/K12 peptides were combined into 8 linear groups, each containing five or six peptides per group (Table24: from top to bottom, light chain peptides are denoted by SEQ ID NOs: 230-250 and heavy chain peptides are denoted by SEQ ID NOs: 251-271).

Table 23: 43 Campath-1H® 15-mer light chain and heavy chain peptides, overlapping by 10 amino acids each

Campath-1H® Peptides

light chain		heavy chain	
Peptide	ID#	Peptide	ID#
DIQMTQSPFSSLSASV	978	QVQLQESGPGGLVLRPS	998
QSPSSLSASVGDRVT	979	ESGPGLVLRPSQTLSTL	999
LSASVGDRVTITCKA	980	LVRPSQTLSTLTCTVS	1000
GDRVTITCKASQNID	981	QTLSTLTCTVSGFTFT	1001
ITCKASQNIDKYLNW	982	TCTVSGFTFTDFYMN	1002
SQNIDKYLNWYQQKP	983	GFTFTDFYMNWVRQP	1003
KYLNWYQQKPGKAPK	984	DFYMNWVRQPPGRGL	1004
YQQKPGKAPKLLIYN	985	WVRQPPGRGLEWIGF	1005
GKAPKLLIYNTNNLQ	986	PGRGLEWIGFIRDKA	1006
LLIYNTNNLQTGVPS	987	EWIGFIRDKAKGYTT	1007
TNNLQTGVPSRFSGS	988	IRDKAKGYTTEYNPS	1008
TGVPSRFSGSGSGTD	989	KGYTTEYNPSVKGRV	1009
RFSGSGSGTDFTFTI	990	EYNPSVKGRVTMLVD	1010
SGSGTDFTFTISLQP	991	VKGRVTMLVDTSKNQ	1011
FTFTISLQPEDIAT	992	TMLVDTSKNQFSLRL	1012
SSLQPEDIATYYCLQ	993	TSKNQFSLRLSSVTA	1013
EDIATYYCLQHISRP	994	FSLRLSSVTAADTAV	1014
YYCLQHISRPRTFGQ	995	SSVTAADTAVYYCAR	1015
HISRPRTFGQGTKVE	996	ADTAVYYCAREGHTA	1016
RTFGQGTKVEIKRTV	997	YYCAREGHTAAPFDY	1017
		EGHTAAPFDYWGQGS	1018
		APFDYWGQGS�VTVS	1019
		WGQGS�VTVSSASTK	1020

Table 24: 42 12G6-SFD1/K12 15-mer light chain and heavy chain peptides, overlapping by 10 amino acids each

12G6-SFD1/K12 Peptides

light chain		heavy chain	
Peptide	ID#	Peptide	ID#
DIVMTQTPLSLSVTP	1027	EVQLVESGGGLVQPG	1048
QTPLSLVTPGQPAS	1028	ESGGGLVQPGGSLRL	1049
LSVTPGQPASISCKS	1029	LVQPGGSLRLSCAAS	1050
GQPASISCKSSQSLL	1030	GSLRLSCAASGFPFS	1079
ISCKSSQSLLYSNGK	1031	SCAASGFPFSNYWMN	1080
SQSLLYSNGKTYLNW	1032	GFPFSNYWMNWVRQA	1081
YSNGKTYLNWVLQKP	1072	NYWMNWVRQAPGKGL	1082
TYLNWVLQKPGQSPQ	1073	WVRQAPGKGLEWVGQ	1055
VLQKPGQSPQRLIYL	1074	PGKGLEWVGQIRLKS	1056
GQSPQRLIYLVSKLD	1036	EWVGQIRLKSNNYAT	1060
RLIYLVSKLDGVPD	1037	IRLKSNNYATHYAES	1061
VSKLDGVPDRFSGS	1038	NNYATHYAESVKGRF	1062
SGVPDRFSGSGSGTD	1039	HYAESVKGRFTISR	1063
RFSGSGSGTDFTLKI	1040	VKGRFTISRDDSKNS	1064
SGGTDFTLKISRVEA	1041	TISRDDSKNSLYLQM	1065
FTLKISRVEAEDVGV	1042	DSKNSLYLQMNSLKT	1066
SRVEAEDVGVYVCVQ	1043	LYLQMNSLKTEDTAV	1067
EDVGYYCVQGSHFH	1075	NSLKTEDTAVYYCTP	1068
YYCVQGSHFHTFGQG	1076	EDTAVYYCTPIDYWG	1083
GSHFHTFGQGTKLEI	1077	YYCTPIDYWGQGTTV	1084
TFGQGTKLEIKRTVA	1078	IDYWGQGTTVTVSSA	1085

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In vitro stimulation

[0544] **DC antigen pulsing and maturation:** Before treatment with the peptides, DCs were thawed, washed and plated in RPMI (Invitrogen, Carlsbad, CA) supplemented with 5% Human Serum (HS, Sigma, St. Louis, MO), 1% Penicillin-Streptomycin (Invitrogen, Carlsbad, CA), 100 ng/ml GM-CSF, and 20 ng/ml IL-4. DCs were plated at 2×10^5 cells/ml in 4 ml media in 6-well tissue culture plates and allowed to adhere for 1 hour at 37°C. Following cell adherence, 10 µg/ml (40 µg total) of each peptide were added to wells containing DCs, correlating to either 120 µg or 160 µg of total peptides added to each well (Campath-1H® 3-peptide or 4-peptide groups), or 200 µg or 240 µg of total peptide added to each well (12G6-SFD1/K12 5-peptide or 6-peptide groups). 40 µg of the pan-DR binding epitope (PADRE) were added to one well of DCs and served as a positive control, as it can bind to most HLA-DR molecules (Alexander J, et al., "Development of high potency universal DR-restricted helper epitopes by modification of high affinity DR-blocking peptides," *Immunity*, 1:751-761 (1994)). Likewise, 40 µg of each of

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three HLA-DR binding Tetanus toxoid peptides (DTIMMEPPYCKGLDIYYKA (SEQ ID NO: 183), SAMLTNLIIFGPGPVLNKNEV (SEQ ID NO: 184), and NNFTVSFWLRVPKVSASHLE (SEQ ID NO: 185)) were added to one well of DCs. Similarly, a heat inactivated adenovirus was employed as a positive antigen source and was added to one well of DCs at 1 µg/ml. Lastly, one group of DCs remained unpulsed with antigen and served as a 'null' educated group. The DCs pulsed with the various antigens were incubated for at least three hours at 37°C. DCs were then treated with a 'maturing cytokine cocktail' containing 50 ng/ml TNF-α, 10 ng/ml IL-6, 25 ng/ml IL-1β (Peprotech, Rocky Hill, NJ) and 500 ng/ml PGE-2 (Sigma Aldrich, St. Louis, MO). The antigen pulsed DCs were then allowed to mature overnight at 37°C.

[0545] Establishment of co-culture: Following peptide loading and maturation, DCs were washed twice with PBS and replenished with 4 ml RPMI supplemented with 10% HS. Autologous CD4⁺ T cells were thawed and resuspended at 2×10^6 cells/ml in RPMI supplemented with 10% HS, Penicillin, and Streptomycin. The DCs were then cultured with naïve CD4⁺ T cells at a 10:1 T cell:DC ratio (8×10^6 T cells: 8×10^5 DCs) in 8 mls media. The co-culture was then incubated at 37°C for 7 days. Approximately 72 hours after initiation of co-culture, the cells were supplemented with 25 IU recombinant IL-2 (Peprotech, Rocky Hill, NJ), and further supplemented with 25 IU recombinant IL-2 in fresh media every 3-4 days thereafter.

[0546] Restimulation of co-culture: At day 7 (Stim #2) and day 14 (Stim #3), the co-cultures were restimulated following the above procedure.

[0547] Proliferation assay: DCs were plated, antigen pulsed and matured as stated above at 5×10^5 cells/ml in 1 ml media on 24-well low binding plates to ease the subsequent transfer of cells to U-bottom assay plates. An irrelevant HLA-DR binding peptide, CS 378-398 (peptide sequence DIEKKIAKMEKASSVFNVNS (SEQ ID NO: 186)), was used as a negative control (Alexander J, et al., "Development of high potency universal DR-restricted helper epitopes by modification of high affinity DR-blocking peptides," *Immunity*, 1:751-761 (1994)). Following 24 hour DC maturation, the cells were detached from plates using ice cold PBS washes. DCs were plated in U-bottom 96 well plates with the antigen stimulated T cells at a 1:1 T cell:DC ratio (2.5×10^4 DC/well). Each T cell group was assayed in triplicate with DC pulsed with the educating peptide(s) (specific response) and DC pulsed with irrelevant peptide (nonspecific response), as well as T cell only and DC only controls. The assay proceeded for 72 hours prior to the addition of 1 µCi tritiated thymidine per well (Perkin Elmer, Waltham, MA). Cells were

harvested on a 96 well plate harvester (Perkin Elmer) and the amount of tritiated thymidine incorporated quantified by measuring CPM on a Wallac Microbeta Trilux counter (Perkin Elmer). The stimulation index was calculated by dividing the specific CPM by the nonspecific CPM.

- 5 [0548] *T Cell Receptor (TCR) V beta usage:* Any CD4+ T cells remaining after establishment of the proliferation assay were frozen for eventual determination of T cell receptor V beta chain expression. Cells were thawed and stained with antibodies recognizing 24 conjugated Vbeta family members for 30 minutes following manufacturer's directions in the IOTest Beta Mark Kit (Beckman Coulter, France). After washing with PBS and resuspending in 10 1% formaldehyde, cells were analyzed on FACScalibur (Becton Dickinson, Franklin Lakes, NJ). The percentage of cells expressing each of the detected V-beta chains was calculated, as summarized in FIG. 102 and FIG. 103.

Campath-1H® Immunogenicity Assessment

- [0549] Immunogenicity assessment of Campath-1H® peptides was performed as described above using PBMCs from ten normal donors, from BioMedSupply (BMS). The summary of the responses as indicated by the stimulation index are depicted in Table 25A. Each donor is listed on one column, and each row lists the group of peptides used to stimulate CD4+ T cells. The Stimulation index (SI) is determined by dividing the specific immune response to the educating peptide group by an irrelevant response. SI values < 2.0 are not listed. The proliferation data 20 for each of the ten donors summarized in Table 25A is reported in FIG. 104A-J. Six donors exhibited a stimulation index greater than 2.0, and as a result were termed 'Campath-1H® responders'. Educated CD4+ T cells from one of the responders, BMS352, exhibited specific immune responses when assayed with two different peptide groups. A seventh donor, BMS486, was also classified as 'responder'. In this donor, a stimulation index 1.7 times background was 25 recorded with the light chain peptide group 986-989. When assessing the V beta upregulation in the educated T cell cultures within this donor, it was shown that the 986-989 educated T cells exhibited high upregulation of a single V beta, Vβ3 (Figure 102). The upregulation of a single V beta and specific proliferative response indicated that BMS486 was a Campath-1H® responder. The three non-responding donors, BMS200, BMS154, and BMS167, did not show 30 proliferative data or V beta upregulation, indicating that a peptide specific immune response did not occur. The Campath-1H® data was quantified as a 70% (7/10) responder rate. The total

number of peptide groups eliciting an immune response was eight. Three of those eight immunogenic peptide groups elicited strong responses in the respective donors with stimulation indices of 3.0 or above (Table 26).

Table 25: Summary of Stimulation Index Data

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Table 25A. Campath-1H® Stimulation Index

Campath-1H® Stimulation Index	BMS200	BMS301	BMS154	BMS484	BMS362	BMS486	BMS150	BMS167	BMS170	BMS352
982, 983, 984 & 987										
988, 989 & 990										
985, 986, 991 & 992		2.6								
993, 994, 995 & 996										
997, 998 & 999						nd				
978, 979, 980, 981										
982, 983, 984, 985									2.0	
986, 987, 988, 989				2.1		1.7				
990, 991, 992, 993										2.1
994, 995, 996, 997										
998, 999, 1000, 1001										
1002, 1003, 1004, 1005										
1006, 1007, 1008, 1009							4.2			5.4
1010, 1011, 1012, 1013					3.0					
1014, 1015, 1016, 1017										
1018, 1019, 1020										
PADRE	2.0			2.0		2.5	2.5	2.8	10.5	2.6
Tetanus	11.2					2.3	4.5		2.3	25.9
Ad-Bgal-HI	27.6	4.5	2.6	3.1	13.0	3.8	24.2	44.5	46.6	3.2
Null										

Table 25B: 12G6-SFD1/K12 Stimulation Index

12G6-SFD1/K12 Stimulation Index	BMS484	BMS486	BMS656	BMS640	BMS361	BMS165	BMS902	BMS928	BMS927	BMS963
1027, 1028, 1029, 1030, 1031										
1032, 1072, 1073, 1074, 1036	2.1								2.5	
1037, 1038, 1039, 1040, 1041										
1042, 1043, 1075, 1076, 1077, 1078										
1048, 1049, 1050, 1079, 1080										
1081, 1082, 1055, 1056, 1060	2.1									
1061, 1062, 1063, 1064, 1065	2.0								nd	
1066, 1067, 1068, 1083, 1084, 1085								2.0		
PADRE	3.0	3.6	2.2	9.8	2.4		2.3		4.7	
TT-974, 975, 976	2.9	5.2		3.2	5.7	12.9	3.7	4.3	22.4	
Ad-Bgal	17.6	11.0	10.9	31.7	29.1		29.3	10.3	8.4	6.3
Null										

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Example 70: Assessment of CD4+ T Cell Responses induced by 12G6-SFD1/K12

[0550] Immunogenicity assessment and V beta analysis of the variable region of 12G6-SFD1/K12 were performed as described in Example 69 for Campath-1H®, employing cells from ten normal donors. The proliferation data for each of the ten donors summarized in Table 25B is reported in FIG. 105A-J. Two of these ten donors were also used in the Campath-1H® assessment described above (BMS486 and BMS484), while the remaining eight donors were tested only with the 12G6-SFD1/K12 peptides. One donor, BMS484, responded to three peptide groups and was classified as a '12G6-SFD1/K12 responder' (Table 25B). Two donors, BMS927

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and BMS928, each responded to one group of peptides and were therefore also classified as responders. Donor BMS928 showed a weak stimulation index of 2.0 to the group containing heavy chain peptides 1066, 1067, 1068, 1083, 1084, and 1085. This response was confirmed by analyzing the proliferative T cells for V beta usage. The responding BMS928 T cells exhibited an upregulation of a single V beta, V β 20 (FIG. 103). Donor BMS927 showed a stimulation index of 2.5 in T cells educated with one group of light chain peptides. V beta analysis of the responding BMS927 T cells did not indicate a single V beta upregulation over background. However, this donor remains in the 'responder' category, as the V beta kit represents only 70% of all possible V beta usages. The 12G6-SFD1/K12 rate of immunogenicity in these 10 donors was 30% (3/10), less than half the rate of Campath-1H® responders (70%). A total of five peptide groups elicited a response, while none of those five groups resulted in a stimulation index greater than 3.0 (Table 26).

Table 26: Summary of Campath-1H® and 12G6-SFD1/K12 immune responses

	Campath-1H®	12G6-SFD1/K12
Percentage of responders	70% (7/10)	30% (3/10)
Number of peptide groups eliciting response	8	5
Responding peptide groups with Stimulation Index $\geq$ 3.0	3/8 (38%)	0/3 (0%)

Summary

[0551] Peptides correlating to the heavy and light chain variable regions of humanized anti-CD52 monoclonal antibody 12G6-SFD1/K12 induced fewer immune responses from ten donors (30%) than peptides from the heavy and light chain variable regions of Campath-1H® (70%). The CD4+ T cell based immune responses that were generated with 12G6-SFD1/K12 were also of less magnitude than the Campath-1H® induced responses.

[0552] The following table lists the sequence identification numbers used herein.

Table 26: List of SEQ ID NOs

SEQ ID NO	TYPE	DESCRIPTION
1	light chain variable	Campath-1G®

SEQ ID NO	TYPE	DESCRIPTION
2	region (VL)	CF1D12
3		8G3 (mouse)
4		4G7 (mouse)
5		9D9 (mouse)
6		11C11 (mouse)
7		3G7 (mouse)
8		5F7 (mouse)
9		12G6 (mouse)
10		23E6 (mouse)
11		2C3 (mouse)
12		7F11 (mouse)
13		4B10 (mouse)
14	heavy chain variable region (VH)	Campath-1G®
15		CF1D12
16		8G3 (mouse)
17		4G7 (mouse)
18		9D9 (mouse)
19		11C11 (mouse)
20		3G7 (mouse)
21		5F7 (mouse)
22		12G6 (mouse)
23		23E6 (mouse)
24		2C3 (mouse)
25		7F11 (mouse)
26		4B10 (mouse)
27	light chain CDR-1	Campath-1H®
28		CF1D12 (mouse)
29		8G3, 4G7, 9D9, 11C11, 3G7 (mouse)
30		5F7 (mouse)
31		12G6, 23E6, 2C3 (mouse)
32		7F11 (mouse)
33		4B10 (mouse)
34	light chain CDR-2	Campath-1H®
35		CF1D12 (mouse)
36		8G3, 11C11, 12G6, 23E6, 2C3 (mouse)

SEQ ID NO	TYPE	DESCRIPTION
37		4G7 (mouse)
38		9D9 (mouse)
39		3G7 (mouse)
40		5F7 (mouse)
41		7F11, 4B10 (mouse)
42	light chain CDR-3	Campath-1H®
43		CF1D12, 8G3, 4G7, 9D9, 11C11, 3G7, 5F7 (mouse)
44		12G6 (mouse)
45		23E6 (mouse)
46		2C3 (mouse)
47		7F11 (mouse)
48		4B10 (mouse)
49	heavy chain CDR-1	Campath-1H®
50		CF1D12, 4G7, 9D9, 11C11, 3G7 (mouse)
51		8G3 (mouse)
52		5F7 (mouse)
53		12G6 (mouse)
54		23E6 (mouse)
55		2C3 (mouse)
56		7F11, 4B10 (mouse)
57	heavy chain CDR-2	Campath-1H®
58		CF1D12 (mouse)
59		8G3 (mouse)
60		4G7 (mouse)
61		9D9, 11C11, 5F7 (mouse)
62		3G7 (mouse)
63		12G6, 2C3 (mouse)
64		23E6 (mouse)
65		7F11 (mouse)
66		4B10 (mouse)
67	heavy chain CDR-3	Campath-1H®
68		CF1D12, 9D9 (mouse)
69		8G3, 4G7, 11C11, 3G7 (mouse)
70		5F7 (mouse)
71		12G6, 23E6 (mouse)

SEQ ID NO	TYPE	DESCRIPTION
72		2C3 (mouse)
73		7F11 (mouse)
74		4B10 (mouse)
75	light chain primers	Lead-ML kappa (forward primer in leader sequence)
76		FR1-ML kappa (forward primer in the framework 1)
77		ML kappa const (reverse primer in constant region)
78		VK-MK (forward primer in the framework 1)
79		MKC-Const (reverse primer in constant region)
80	heavy chain primers	MH-SP-ALT1 (forward primer in leader sequence)
81		MH-SP-ALT2 (forward primer in leader sequence)
82		MH-FR1 (forward primer in the framework 1)
83		MH-FR1-1 (forward primer in the framework 1)
84		MH-J2 (reverse primer in J region)
85		MH-gamma-const (reverse primer in constant region)
86		VH MH1 (forward primer in the framework 1)
87		VH MH2 (forward primer in the framework 1)
88		VH MH3 (forward primer in the framework 1)
89		VH MH4 (forward primer in the framework 1)
90		VH MH5 (forward primer in the framework 1)
91		VH MH6 (forward primer in the framework 1)
92		VH MH7 (forward primer in the framework 1)
93		IgG1 (reverse primer in mouse IgG1 CH1 constant region)
94		IgG2A (reverse primer in mouse IgG2A CH1 constant region)
95		IgG2B (reverse primer in mouse IgG2B CH1 constant region)
96	VH (partial)	4B10 (mouse): alignment
97	human germline (VH)	VH3-72: alignment
98	VH (partial)	4B10 (humanized): alignment
99	mouse VL (partial)	4B10 (mouse): alignment
100	human germline (VL)	VK2-A18b: alignment
101	VL (partial)	4B10 (humanized): alignment
102	VL	4B10-VK1 (humanized)
103	VH	4B10-VH1 (humanized)

SEQ ID NO	TYPE	DESCRIPTION	
104	CD52 alanine-scanning mutant peptides	WT	
105		MUT 1	
106		MUT 2	
107		MUT 3	
108		MUT 4	
109		MUT 5	
110		MUT 6	
111		MUT 7	
112		MUT 8	
113		MUT 9	
114		MUT 10	
115	LC CDR-1	K/RSSQSLL/V/IXS/TN/DGXS/TYLY	
116		K/RSSQSLL/V/IHS/TNGXS/TYLY	
117		RSSQSLVHTNGNS/TYLY	
118	LC CDR-2	XVSXXXS	
119		XVSXRXS	
120		MVSXRFS	
121	LC CDR-3	XQXXH/R/KF/L/V/IXX	
122		SQSXH/R/KF/L/V/IPX	
123		SQSXHVPF/P	
124	HC CDR-1	GFXFXXYW/YMX	
125		GFTFXXYW/YMX	
126		GFTFTDYW/YMS	
127	HC CDR-2	XIRXKXBXYXTXYXXSVKG	
128		XIRXKXNYTTEYXXSVKG	
129		FIRNKANGYTTEYXXSVKG	
130	HC CDR-3	TXXXY/F/W	
131		TRYXY/F/WFDY	
132		TRYIF/WFDY	
133	JH6	WGQGTTVTVSS	
134	JK2	FGQGTKLEIK	
135	JK5	FGQGTRLEIK	
136	VH	SFD1	7F11
137		SFD2	

SEQ ID NO	TYPE	DESCRIPTION	
138	VL	VK2	
139	VH	SFD1	2C3
140		12	
141		15	
142		16	
143		17	
144		19	
145	VL	VK1	2C3
146		VK11	
147		VK12	
148		VK13	
149	VH	SFD1	12G6
150		VH10	
151		VH11	
152		VH12	
153	VL	VK1	
154		VK10	
155		VK11	
156		VK12	
157		VK13	
158	VH	VH10	9D9
159		VH11	
160		VH15	
161		VH16	
162		VH17	
163		VH18	
164	VL	VK2	
165		VK12	
166		VK13	
167		VK14	
168		VK15	
169	CD52 alanine-scanning peptides	MUT 1	
170		MUT 2	
171		MUT 3	

SEQ ID NO	TYPE	DESCRIPTION	
172		MUT 4	
173		MUT 5	
174		MUT 6	
175		MUT 7	
176		MUT 8	
177		MUT 9	
178		MUT 10	
179		MUT 11	
180		MUT 12	
181		WT1	
182		WT2	
183	Tetanus toxoid HLA-DR-binding peptides	DTIMMEPPYCKGLDIYYKA	
184		SAMLTNLIIFGPGPVLNKNEV	
185		NNFTVSFWLRVPKVSASHLE	
186	“irrelevant” HLA-DR-binding peptide	CS 378-398	
187	Campath-1H® LC overlapping 15-mer peptides for immunogenicity study	978	
188		979	
189		980	
190		981	
191		982	
192		983	
193		984	
194		985	
195		986	
196		987	
197		988	
198		999	
199		990	
200		991	
201		992	
202		993	
203		994	
204		995	
205		996	
206		997	

SEQ ID NO	TYPE	DESCRIPTION	
207	Campath-1H® HC overlapping 15-mer peptides for immunogenicity study	998	
208		999	
209		1000	
210		1001	
211		1002	
212		1003	
213		1004	
214		1005	
215		1006	
216		1007	
217		1008	
218		1009	
219		1010	
220		1011	
221		1012	
222		1013	
223		1014	
224		1015	
225		1016	
226		1017	
227		1018	
228		1019	
229		1020	
230	12G6-SFD1/K12 LC overlapping 15-mer peptides for immunogenicity study	1027	
231		1028	
232		1029	
233		1030	
234		1031	
235		1032	
236		1072	
237		1073	
238		1074	
239		1036	
240		1037	
241		1038	
242		1039	

SEQ ID NO	TYPE	DESCRIPTION	
243		1040	
244		1041	
245		1042	
246		1043	
247		1075	
248		1076	
249		1077	
250		1078	
251	12G6-SFD1/K12 HC overlapping 15-mer peptides for immunogenicity study	1048	
252		1049	
253		1050	
254		1079	
255		1080	
256		1081	
257		1082	
258		1055	
259		1056	
260		1060	
261		1061	
262		1062	
263		1063	
264		1064	
265		1065	
266		1066	
267		1067	
268		1068	
269		1083	
270		1084	
271		1085	
272	HC	2C3-SFD1	2C3
273	LC	2C3-K12	
274	HC	7F11-SFD1	7F11
275	LC	7F11-K2	
276	HC	9D9-H16	9D9
277		9D9-H18	
278	LC	9D9-K13	

SEQ ID NO	TYPE	DESCRIPTION	
279	HC	12G6-SFD1	12G6
280	LC	12G6-K12	
281	HC	4B10-H1	4B10
282	LC	4B10-K1	
283	HC (nucleic acid)	2C3-SFD1	2C3
284	LC (nucleic acid)	2C3-K12	
285	HC (nucleic acid)	7F11-SFD1	7F11
286	LC (nucleic acid)	7F11-K2	
287	HC (nucleic acid)	9D9-H16	9D9
288		9D9-H18	
289	LC (nucleic acid)	9D9-K13	
290	HC (nucleic acid)	12G6-SFD1	12G6
291	LC (nucleic acid)	12G6-K12	
292	HC (nucleic acid)	4B10-H1	4B10
293	LC (nucleic acid)	4B10-K1	
294	HC CDR-3	7F11-SFD2 (ARYIFFDY)	7F11

[0553] The teachings of all patents, published applications and references cited herein are incorporated by reference in their entirety.

- [0554] While this invention has been particularly shown and described with references to
5 example embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An isolated monoclonal anti-human CD52 antibody or an antigen-binding portion that binds to human CD52, wherein the light chain and heavy chain of said antibody comprise the three complementarity determining regions (CDRs) found in SEQ ID NOs: 9 and 22, respectively.
2. An isolated monoclonal anti-human CD52 antibody or an antigen-binding portion that binds to human CD52, wherein the heavy chain and the light chain of said antibody comprise the amino acid sequences of SEQ ID NOs: 279 and 280, respectively, without the signal sequences.
3. The isolated monoclonal anti-human CD52 antibody or an antigen-binding portion of claim 1, wherein the light chain of said antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 153-157.
4. The isolated monoclonal anti-human CD52 antibody or an antigen-binding portion of claim 1, wherein the light chain of said antibody comprises the amino acid sequence of SEQ ID NO: 280.
5. The isolated monoclonal anti-human CD52 antibody or antigen-binding portion of claim 1, wherein the heavy chain of said antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 149-152.
6. The isolated monoclonal anti-human CD52 antibody or antigen-binding portion of claim 1, wherein the heavy chain of said antibody comprises an amino acid sequence of SEQ ID NO: 279.
7. The isolated monoclonal antibody or antigen-binding portion of any one of claims 1-6, wherein the antibody is a humanized antibody, a mouse antibody, or a chimeric antibody.

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8. The isolated monoclonal antibody or antigen-binding portion of any one of claims 1-7, wherein the framework regions of the heavy chain utilize a VH3-72 or VH3-23 human germline sequence, and wherein the framework regions of the light chain utilize a VK2 A18b human germline sequence.
9. The isolated monoclonal antibody or antigen-binding portion of any one of claims 1-8, wherein the antibody comprises heavy chain (H)-CDR1, H-CDR2, H-CDR3, and light chain (L)-CDR1, L-CDR2, and L-CDR3 whose amino acid sequences are SEQ ID NOs: 53, 63, 71, 31, 36, and 44, respectively.
10. The isolated monoclonal antibody or antigen-binding portion of any one of claims 1-9, wherein the light chain and heavy chain of said antibody comprise the amino acid sequences of SEQ ID NOs: 9 and 22, respectively.
11. The isolated monoclonal antibody or antigen-binding portion of any one of claims 1-10, wherein said heavy chain and light chain comprise the amino acid sequences of SEQ ID NOs: 149 and 155, respectively; or SEQ ID NOs: 149 and 156, respectively.
12. The isolated monoclonal antibody of any one of claims 1-11, wherein said antibody is an IgG1, IgG2, IgG3, or IgG4 molecule.
13. The antigen-binding portion of any one of claims 1-11, wherein said portion is a single chain antibody, Fv, Fab, Fab', F(ab')₂, Fd, single chain Fv molecule (scFv), bispecific single chain Fv dimer, diabody, domain-deleted antibody or single domain antibody (dAb).
14. The isolated monoclonal antibody or antigen-binding portion of any one of claims 1-13, wherein said antibody or portion binds to an amino acid sequence comprising SEQ ID NO: 104, and optionally, the binding of said antibody or portion to SEQ ID NO: 104 is reduced by an alanine substitution at one or more of residues 4, 7, 8, and 11 of SEQ ID NO: 104.

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15. The isolated monoclonal antibody or antigen-binding portion of any one of claims 1-14, wherein said antibody or antigen-binding portion has one or more properties selected from the group consisting of:

- a) depletes T or B lymphocytes, or both;
- b) preferentially depletes T lymphocytes as compared to B lymphocytes;
- c) increases circulating serum levels of TNF-alpha, IL-6, or MCP-1;
- d) mediates antibody-dependent cell mediated cytotoxicity (ADCC) of CD52-expressing cells;
- e) mediates complement-dependent cytotoxicity (CDC) of CD52-expressing cells;
- f) binds to human CD52 in the presence of neutralizing antibodies to alemtuzumab; and
- g) promotes intracellular signaling in human T or B lymphocytes, or both.

16. A composition comprising the isolated monoclonal antibody or antigen-binding portion of any one of claims 1-15, and a pharmaceutically acceptable vehicle or carrier.

17. An isolated nucleic acid encoding the heavy chain, or the light chain, or both, of an antibody or antigen-binding portion of any one of claims 1-15.

18. The isolated nucleic acid of claim 17, wherein said isolated nucleic acid comprises:

- a) a heavy chain nucleotide sequence of SEQ ID NO: 290, or said nucleotide sequence without the sequence encoding a signal peptide;
- b) a light chain nucleotide sequence of SEQ ID NO: 291, or said nucleotide sequence without the sequence encoding a signal peptide.

19. The isolated nucleic acid of claim 18, wherein said isolated nucleic acid comprises a heavy chain nucleotide sequence and a light chain nucleotide sequence selected from the group consisting of SEQ ID NO: 290 and SEQ ID NO: 291, respectively, both without sequences encoding signal peptides.

20. A host cell comprising a first nucleic acid sequence encoding the heavy chain of an isolated monoclonal antibody or antigen-binding portion of any one of claims 1-15, said first nucleic acid sequence operably linked to an expression control element, and a second

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nucleic acid sequence encoding the light chain of said monoclonal antibody or antigen-binding portion, said second nucleic acid sequence operably linked to an expression control element.

21. A method of expressing an anti-human CD52 antibody or an antigen-binding portion that binds to human CD52, comprising maintaining the host cell of claim 20 under conditions appropriate for expression of the antibody or portion, and expressing said antibody or portion.

22. A method for inducing immunosuppression in a patient, comprising administering to the patient an effective amount of the antibody or antigen-binding portion of any one of claims 1-15.

23. The method of claim 22, wherein the patient has an autoimmune disease.

24. A method for targeting CD52⁺ cells in a cancer patient, comprising administering to the patient an effective amount of the isolated monoclonal antibody or antigen-binding portion of any one of claims 1-15.

25. A method of inhibiting angiogenesis in a patient in need thereof, comprising administering an effective amount of the isolated monoclonal antibody or portion of any one of claims 1-15, to the patient.

26. The method of claim 22, wherein the patient is receiving transplantation.

27. The method of claim 23, wherein said autoimmune disease is multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, or vasculitis.

28. The method of claim 24, wherein the cancer patient has leukemia.

29. The method of claim 24, wherein the cancer patient has lymphoma.

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30. The method of claim 24, wherein the cancer patient has T cell malignancy, and the antibody or portion preferentially depletes T cells as compared to B cells.
31. The method of claim 24, wherein the cancer patient has a solid tumor.
32. The method of claim 22, further comprising administering to the patient a neutrophil or NK cell stimulatory agent.
33. The method of claim 24, further comprising administering to the patient a neutrophil or NK cell stimulatory agent.
34. The method of claim 22, further comprising administering to the patient a T regulatory cell stimulatory agent.
35. The method of claim 24, further comprising administering to the patient a T regulatory cell stimulatory agent.
36. The method of claim 25, wherein the patient has a solid tumor.
37. The method of claim 25, wherein the patient has neovascularization.
38. Use of the antibody or antigen-binding portion of any one of claims 1-15 in preparation of a medicament for inducing immunosuppression in a patient in need thereof.
39. Use of the antibody or antigen-binding portion of any one of claims 1-15 in preparation of a medicament for targeting CD52+ cells in a cancer patient.
40. Use of the antibody or antigen-binding portion of any one of claims 1-15 in preparation of a medicament for inhibiting angiogenesis in a patient in need thereof.

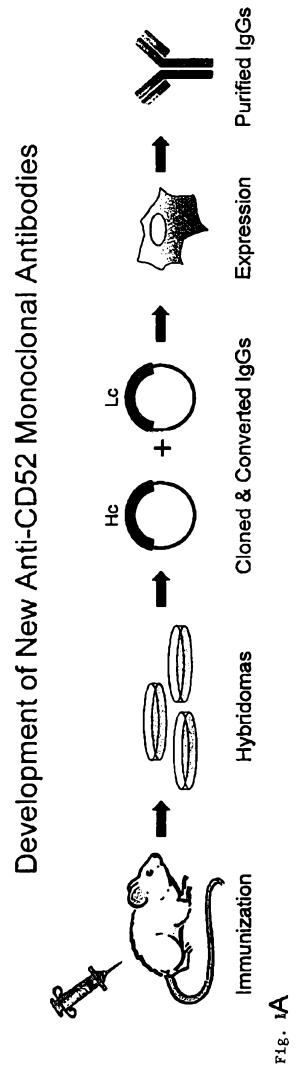


Fig. 1A

mAb	Original isotype
4G7	mgG ₁
9D8	mgG ₁
7F11	mgG ₁
4B10	mgG _{2a}
8G3	mgG ₁
11C11	mgG ₁
3G7	mgG _{2a}
12G8	mgG ₁
5F7	mgG ₁
23E8	mgG ₁
2C3	mgG ₁

Fig. 1B

FIG. 1A - 1B

Anti-CD52 Binders

Light Chain Sequences of Mouse Anti-Human CD52 Antibodies

Light Chain (kappa) sequences

1) Campath 16 Kappa (rat)	[-----FR3-----][CDR1-----][CDR2-----][CDR3-----][REGION-]	(SEQ ID NO:1)
2) Mouse 8C3.25	DIKWTQSPFASVGVNLTCKKASQNDK...YLHWYQKLGESFKLLINTNNLQGTGIPNFSGSGCTDFTLISLQPEVDVATYICQKHSRP.ATFGCTKLEIK	(SEQ ID NO:2)
3) Mouse 8C3.25.3.5 Kappa	DVWMTQPTLASVTHGHPASISCKSSQSLLSDSGKTYLWMLQRPQGSFKRLIYLVNKLDSGVDPFSGSGGCTDFTLKISRVEAEDLGYVYCWQGTTHP.WTFGGGCKLEIK	(SEQ ID NO:3)
4) Mouse 8C3.25.3.5 Kappa	DIVLTQSTLSVYTIQGPASISCKSSQSLLSDSGKTYLWMLQRPQGSFKRLIYLVNKLDSGVDPFSGSGGCTDFTLKISRVEAEDLGYVYCWQGTTHP.WTFGGGCKLEIK	(SEQ ID NO:4)
5) Mouse 8C3.25.3.5 Kappa	DIVLTQSTLSVYTIQGPASISCKSSQSLLSDSGKTYLWMLQRPQGSFKRLIYLVNKLDSGVDPFSGSGGCTDFTLKISRVEAEDLGYVYCWQGTTHP.WTFGGGCKLEIK	(SEQ ID NO:5)
6) Mouse 8C3.25.3.5 Kappa	DIVLTQSTLSVYTIQGPASISCKSSQSLLSDSGKTYLWMLQRPQGSFKRLIYLVNKLDSGVDPFSGSGGCTDFTLKISRVEAEDLGYVYCWQGTTHP.WTFGGGCKLEIK	(SEQ ID NO:6)
7) Mouse 8C3.25.3.5 Kappa	DIVLTQSTLSVYTIQGPASISCKSSQSLLSDSGKTYLWMLQRPQGSFKRLIYLVNKLDSGVDPFSGSGGCTDFTLKISRVEAEDLGYVYCWQGTTHP.WTFGGGCKLEIK	(SEQ ID NO:7)
8) Mouse 8C3.25.3.5 Kappa	DIVLTQSTLSVYTIQGPASISCKSSQSLLSDSGKTYLWMLQRPQGSFKRLIYLVNKLDSGVDPFSGSGGCTDFTLKISRVEAEDLGYVYCWQGTTHP.WTFGGGCKLEIK	(SEQ ID NO:8)
9) Mouse 8C3.25.3.5 Kappa	DIVLTQSTLSVYTIQGPASISCKSSQSLLSDSGKTYLWMLQRPQGSFKRLIYLVNKLDSGVDPFSGSGGCTDFTLKISRVEAEDLGYVYCWQGTTHP.WTFGGGCKLEIK	(SEQ ID NO:9)
10) Mouse 8C3.25.3.5 Kappa	DIVLTQSTLSVYTIQGPASISCKSSQSLLSDSGKTYLWMLQRPQGSFKRLIYLVNKLDSGVDPFSGSGGCTDFTLKISRVEAEDLGYVYCWQGTTHP.WTFGGGCKLEIK	(SEQ ID NO:10)
11) Mouse 8C3.25.3.5 Kappa	DIVLTQSTLSVYTIQGPASISCKSSQSLLSDSGKTYLWMLQRPQGSFKRLIYLVNKLDSGVDPFSGSGGCTDFTLKISRVEAEDLGYVYCWQGTTHP.WTFGGGCKLEIK	(SEQ ID NO:11)
12) Mouse 8C3.25.3.5 Kappa	DIVLTQSTLSVYTIQGPASISCKSSQSLLSDSGKTYLWMLQRPQGSFKRLIYLVNKLDSGVDPFSGSGGCTDFTLKISRVEAEDLGYVYCWQGTTHP.WTFGGGCKLEIK	(SEQ ID NO:12)
13) Mouse 8C3.25.3.5 Kappa	DIVLTQSTLSVYTIQGPASISCKSSQSLLSDSGKTYLWMLQRPQGSFKRLIYLVNKLDSGVDPFSGSGGCTDFTLKISRVEAEDLGYVYCWQGTTHP.WTFGGGCKLEIK	(SEQ ID NO:13)

FIG 2

Heavy Chain Sequences of Mouse Anti-Human CD52 Antibodies

Heavy Chain Sequence	
1) Campath 1G heavy (rat)	(SEQ ID No:14)
2) CF1D12 IgG3 heavy	(SEQ ID No:15)
3) Mouse 8G3.25.3.5 IgG3 heavy	(SEQ ID No:16)
4) Mouse 4G7.F3 IgG3 heavy	(SEQ ID No:17)
5) Mouse 9D9.A2 IgG3 heavy	(SEQ ID No:18)
6) Mouse 11D5.10 IgG3 heavy	(SEQ ID No:19)
7) Mouse 3G7.F9 IgG3b heavy	(SEQ ID No:20)
8) Mouse 5F7.1.1.4 IgG3 heavy	(SEQ ID No:21)
9) Mouse 12G6.15.1.2 IgG3 heavy	(SEQ ID No:22)
10) Mouse 23B6.2.2.1 IgG3 heavy	(SEQ ID No:23)
11) Mouse 2C3.3.8.1 IgG3 heavy	(SEQ ID No:24)
12) Mouse 4B10.1.2.1 IgG3 heavy	(SEQ ID No:25)
13) Mouse 4B10.1.2.4 IgG2a heavy	(SEQ ID No:26)

[-----FR1-----][--CDR1--][-----FR2-----][-----CDR2-----][-----FR3-----][-----CDR3--][--REGION-]	
1) EVKLLEESGGGLVQPGGSKMLSCVSGSFTFTDYMNNIRQPKAGPEMLGFIKDKAKGYTETNPSPWKGRTTISRINTQNNILYQNNLRABDTATYTCAREGHTAAPFDYWGQGVMTVSS	EVKLLEESGGGLVQPGGSKMLSCVSGSFTFTDYMNNIRQPKAGPEMLGFIKDKAKGYTETNPSPWKGRTTISRINTQNNILYQNNLRABDTATYTCAREGHTAAPFDYWGQGVMTVSS
2) EVKLLEESGGGLVQPGGSKMLSCVSGSFTFTSDANNMDWRQSPKGLGWAEIRNKAKNHVATYAESVKGRTTISRDDSKSVYLQNNLRABDTGIYYC..TTL.....DSWQGGTALTIVSS	EVKLLEESGGGLVQPGGSKMLSCVSGSFTFTSDANNMDWRQSPKGLGWAEIRNKAKNHVATYAESVKGRTTISRDDSKSVYLQNNLRABDTGIYYC..TTL.....DSWQGGTALTIVSS
3) EVKLLEESGGGLVQPGGSKMLSCVSGRFTTSDANNMDWRQSPKGLGWAEIRNKAKNHATYAESVKGRTTISRDDSKSRVFLQNNLRPEDTGIYYC..TSL.....DYWGQGGTTLTVSS	EVKLLEESGGGLVQPGGSKMLSCVSGRFTTSDANNMDWRQSPKGLGWAEIRNKAKNHATYAESVKGRTTISRDDSKSRVFLQNNLRPEDTGIYYC..TSL.....DYWGQGGTTLTVSS
4) EVKLLEESGGGLVQPGGSKMLSCVSGSFTTSDANNMDWRQSPKGLGWAEIRNKAKNHVATYAESVKGRTTISRDDSKSVYLQNNLRATEDTGIYYC..TSL.....DYWGQGGTALTIVSS	EVKLLEESGGGLVQPGGSKMLSCVSGSFTTSDANNMDWRQSPKGLGWAEIRNKAKNHVATYAESVKGRTTISRDDSKSVYLQNNLRATEDTGIYYC..TSL.....DYWGQGGTALTIVSS
5) EVKLLEESGGGLVQPGGSKMLSCVSGSFTTSDANNMDWRQSPKGLGWAEIRNKAKNHATYAESVKGRTTISRDDSKSRVYLQNNLRATEDTGIYYC..TTL.....DSWQGGTSTVTVSS	EVKLLEESGGGLVQPGGSKMLSCVSGSFTTSDANNMDWRQSPKGLGWAEIRNKAKNHATYAESVKGRTTISRDDSKSRVYLQNNLRATEDTGIYYC..TTL.....DSWQGGTSTVTVSS
6) EVKLLEESGGGLVQPGGSKMLSCVSGSFTTSDANNMDWRQSPKGLGWAEIRNKAKNHATYAESVKGRTTISRDDSKSVYLQNNLRABDTGIYYC..TSL.....DYWGQGGTTLTVSS	EVKLLEESGGGLVQPGGSKMLSCVSGSFTTSDANNMDWRQSPKGLGWAEIRNKAKNHATYAESVKGRTTISRDDSKSVYLQNNLRABDTGIYYC..TSL.....DYWGQGGTTLTVSS
7) EVKLLEESGGGLVQPGGSKMLSCVSGSFTTSDANNMDWRQSPKGLGWAEIRNKYNNHATYAESVKGRTTISRDDSKSVYLQNNLRABDTGIYYC..TSL.....DYWGQGGTTLTVSS	EVKLLEESGGGLVQPGGSKMLSCVSGSFTTSDANNMDWRQSPKGLGWAEIRNKYNNHATYAESVKGRTTISRDDSKSVYLQNNLRABDTGIYYC..TSL.....DYWGQGGTTLTVSS
8) EVKLLEESGGGLVQPGGSKMLSCVSGLTFTSDANNMDWRQSPKGLGWAEIRNKAKNHATYAESVKGRTTISRDDSKSVYLQNNLRABDTGIYYC..TGL.....DYWGHTGTSVTVSS	EVKLLEESGGGLVQPGGSKMLSCVSGLTFTSDANNMDWRQSPKGLGWAEIRNKAKNHATYAESVKGRTTISRDDSKSVYLQNNLRABDTGIYYC..TGL.....DYWGHTGTSVTVSS
9) EVQLLEESGGGLVQPGGSKMLSCVSGSFTTSDANNMDWRQSPKGLGWAEIRLKSNNYATHYAESVKGRTTISRDDSKSVYLQNNLRABDTGIYYC..TPI.....DYWGQGGTTLTVSS	EVQLLEESGGGLVQPGGSKMLSCVSGSFTTSDANNMDWRQSPKGLGWAEIRLKSNNYATHYAESVKGRTTISRDDSKSVYLQNNLRABDTGIYYC..TPI.....DYWGQGGTTLTVSS
10) EVKLLEESGGGLVQPGGSKMLSCVSGSFTTFTNNKYNNHATYAESVKGRTTISRDDSKSVYLQNNLRABDTGIYYC..IRLKSNNYATHYAESVKGRTTISRDDSKSVYLQNNLRABDTGIYYC..TPV.....DYWGQGGTTLTVSS	EVKLLEESGGGLVQPGGSKMLSCVSGSFTTFTNNKYNNHATYAESVKGRTTISRDDSKSVYLQNNLRABDTGIYYC..IRLKSNNYATHYAESVKGRTTISRDDSKSVYLQNNLRABDTGIYYC..TPV.....DYWGQGGTTLTVSS
11) EVKLLEESGGGLVQPGGSKMLSCVSGSFTTFTDYMNNWRQPPKALEMLGFIKDKANGYTTETNASVKGRTTISRVEQSILYLQNNLRABDSATYYC..TRYIFF..DYWGQGGTTLTVSS	EVKLLEESGGGLVQPGGSKMLSCVSGSFTTFTDYMNNWRQPPKALEMLGFIKDKANGYTTETNASVKGRTTISRVEQSILYLQNNLRABDSATYYC..TRYIFF..DYWGQGGTTLTVSS
13) EVQLLEESGGGLVQPGGALSICVSGSFTTFTDYMNNWRQPPKALEMLGFIKDKANGYTTETNASVKGRTTISRDDSQSILYLQNNLRABDSATYYC..TRYINF..DYWGQGGTTLTVSS	EVQLLEESGGGLVQPGGALSICVSGSFTTFTDYMNNWRQPPKALEMLGFIKDKANGYTTETNASVKGRTTISRDDSQSILYLQNNLRABDSATYYC..TRYINF..DYWGQGGTTLTVSS

FIG 3

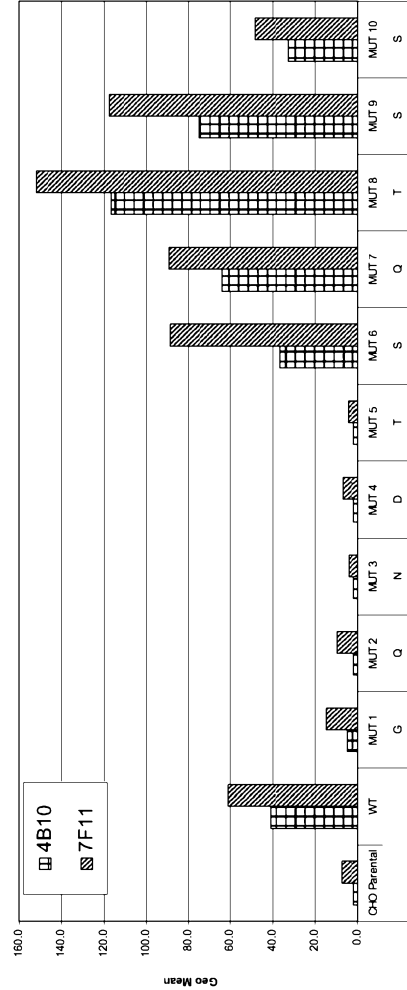
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Point Mutations within the CD52 Protein

WT	GQNDTSQTSSPS
MUT 1	<u>A</u> QNDTSQTSSPS
MUT 2	G <u>A</u> NDTSQTSSPS
MUT 3	GQ <u>A</u> DTSQTSPPS
MUT 4	GQNA <u>T</u> SQTSSPS
MUT 5	GQND <u>A</u> SQTSSPS
MUT 6	GQNDT <u>A</u> QTSSPS
MUT 7	GQNDTS <u>A</u> TSSPS
MUT 8	GQNDTSQ <u>A</u> SSPS
MUT 9	GQNDTSQT <u>A</u> SPS
MUT 10	GQNDTSQTS <u>A</u> PS

FIG. 4

FIG. 5A



N terminal binding

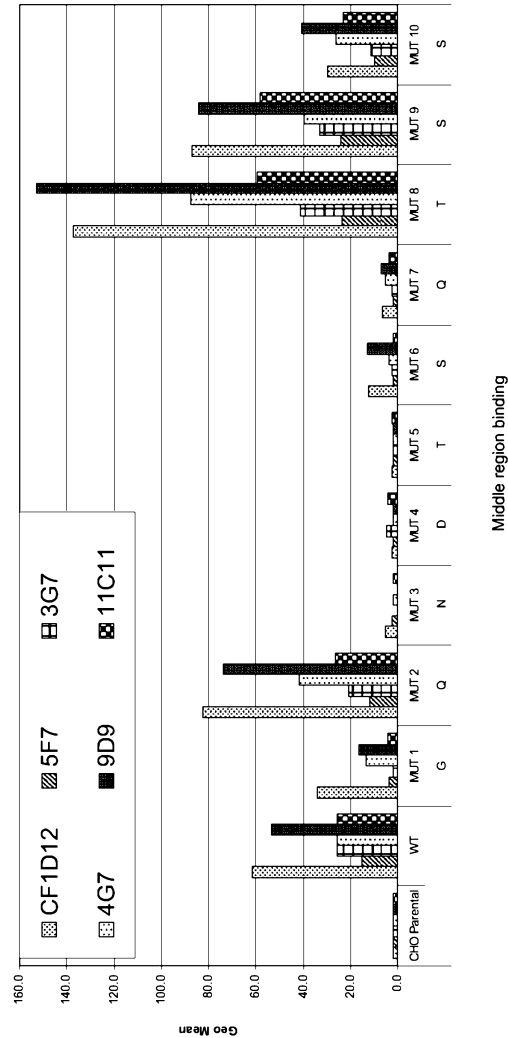


FIG. 5B



FIG. 5C

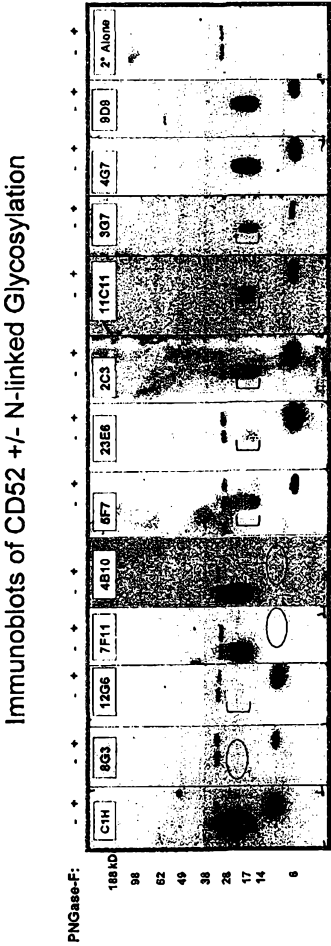


FIG. 5D

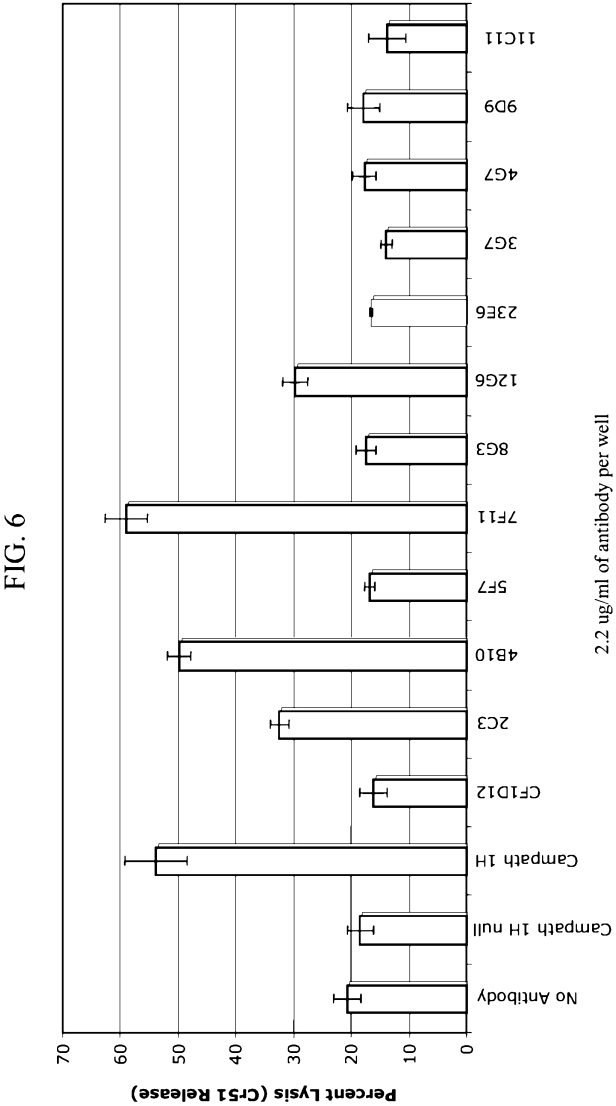


FIG. 7

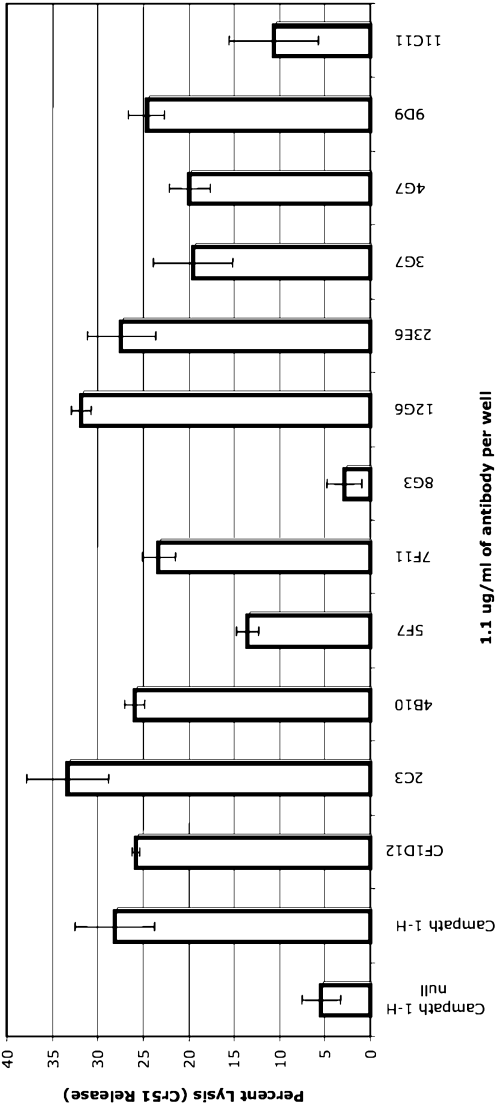


FIG. 8A

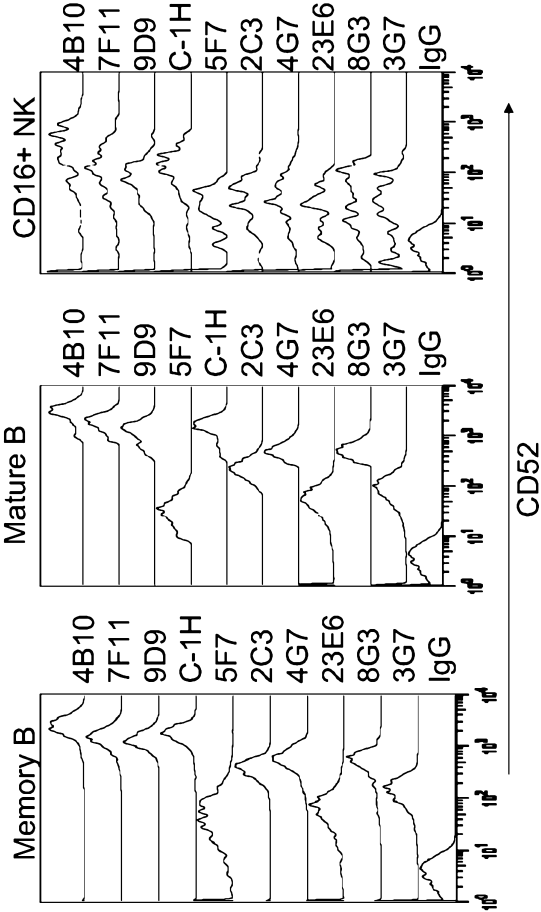


FIG. 8B

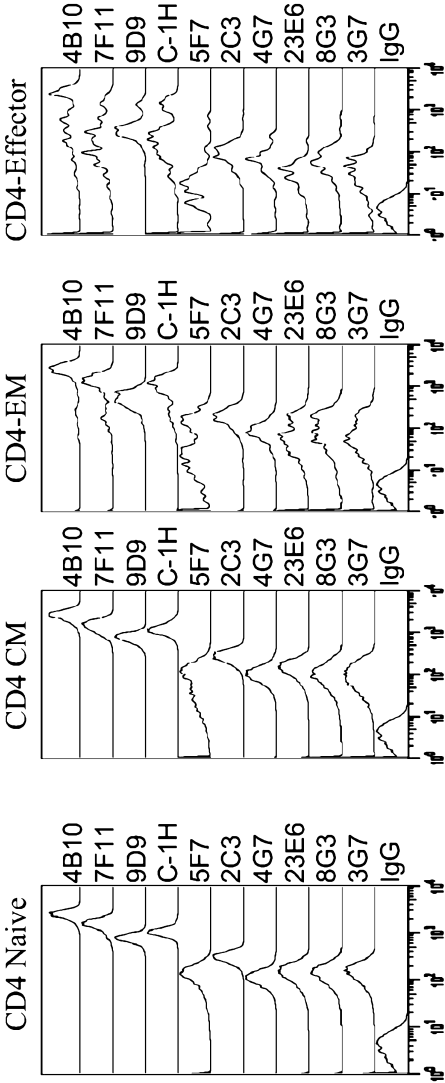
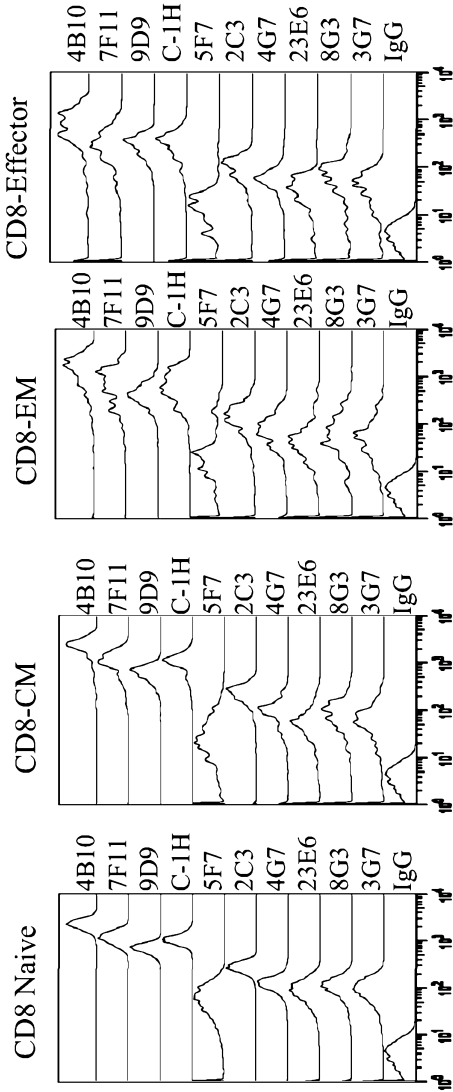


FIG. 8C



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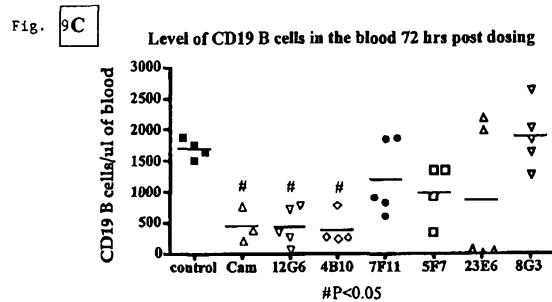
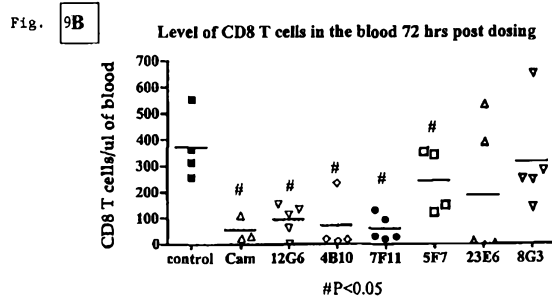
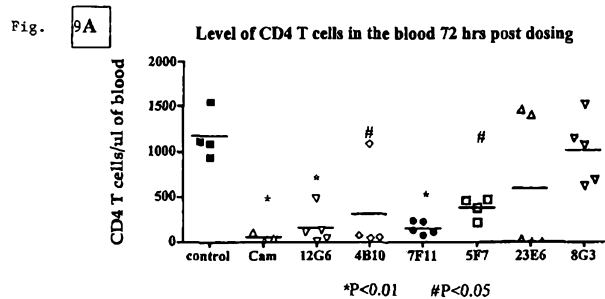
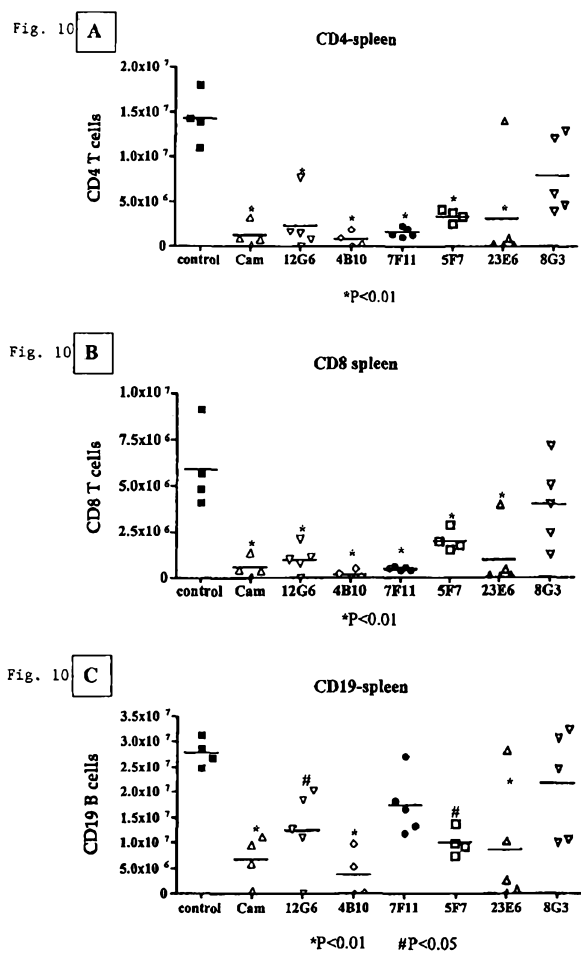


FIG. 9A - 9C

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**FIG. 10a** - 10C

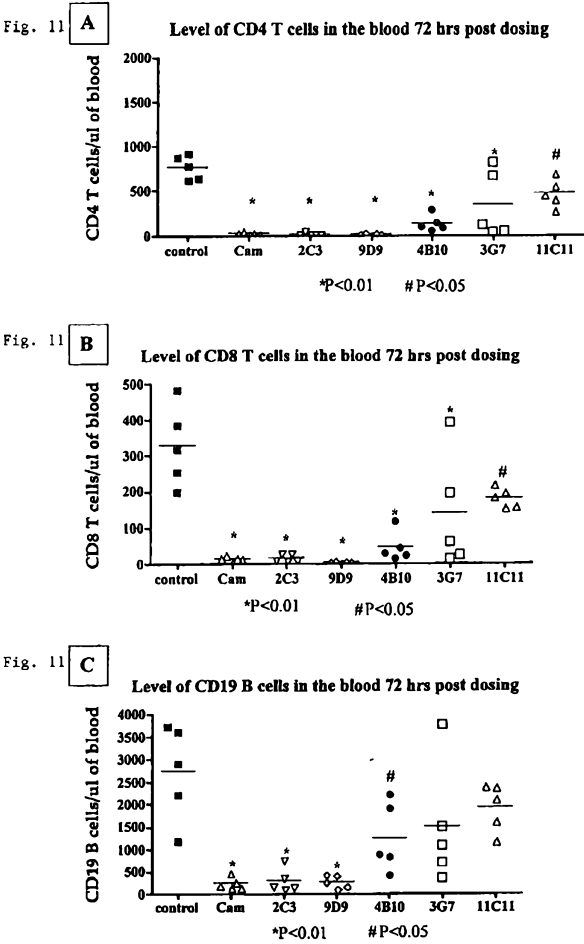


FIG. 11A - 11C

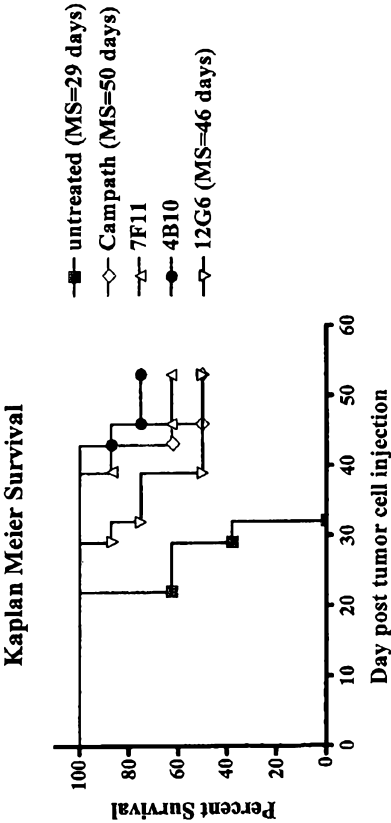


FIG. 12

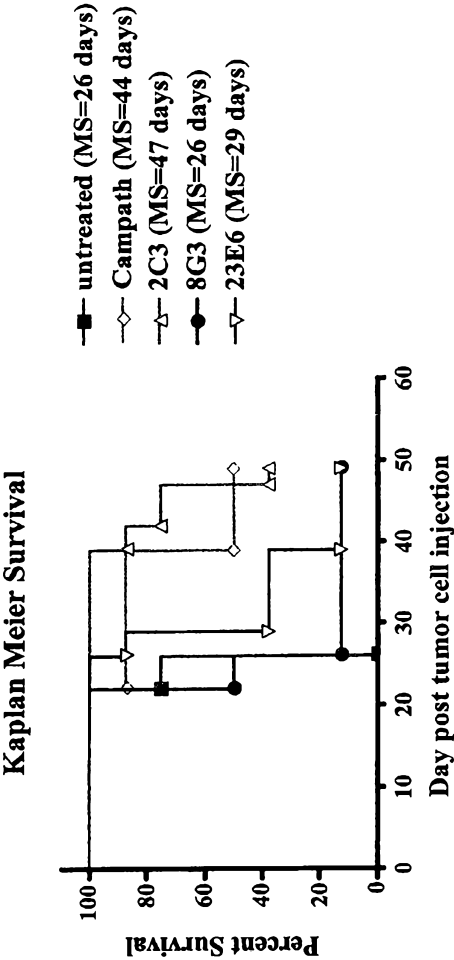


FIG. 13

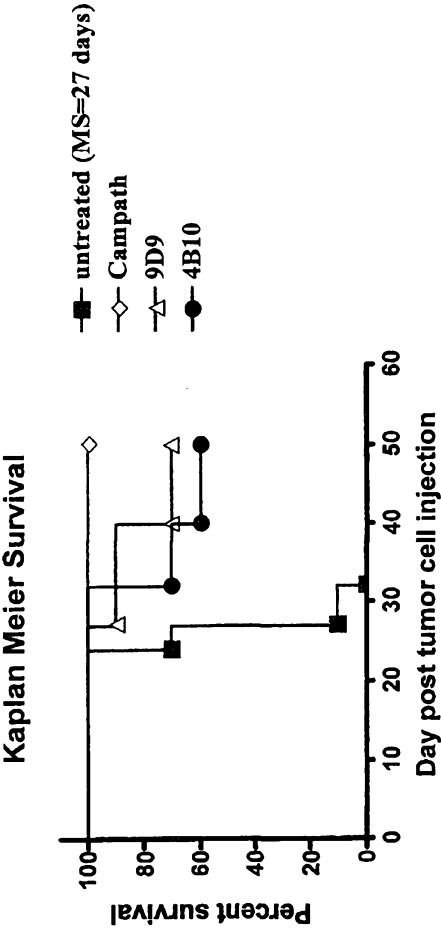


FIG. 14

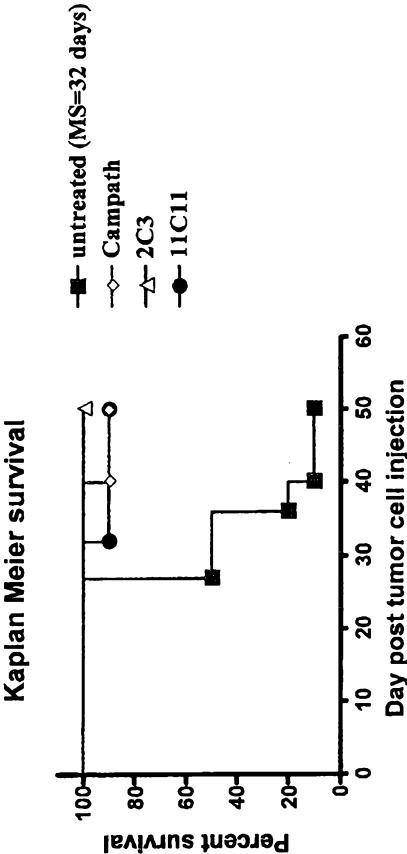


FIG. 15

Alignment of anti human CD52 mouse (m) antibody V region with human germline(h) and humanized anti-CD52 V region sequences

4B10 Heavy Chain V region				
4B10 Heavy chain(m)	1	EVQLVESGGGLVQPGGALSLSCAGSGFTFTDYMSVVRQPPKALEWLG	50	(SEQ ID NO:96)
VH3-72 germline(h)	1	EVQLVESGGGLVQPGGSLRLSCAASGFTESDHMDVVRQAPKGLGWGR	50	(SEQ ID NO:97)
4B10 humanized HC	1	EVQLVESGGGLVQPGGSLRLSCAASGFTFDYINSMVVRQAPKGLGWGF	50	(SEQ ID NO:98)
		*****.*		
		*****.*		
		*****.*		
4B10 Heavy chain(m)	51	IRNKANGYTTEYSASVKGRFTISRDDSQSLYLQMNALRAEDSATYYCTR	100	
VH3-72 germline(h)	51	TRNKANGYTTEYAAASVKGRFTISRDDSKNSLYLQMNLSKTEDTAVYYCAR	100	
4B10 humanized HC	51	IRNKANGYTTEYSASVKGRFTISRDDSKNSLYLQMNLSKTEDTAVYYCAR	100	
		*****.*		
		*****.*		
		*****.*		
4B10 Light chain V region				
4B10 Light chain(m)	1	DIVMTQSPPLSLTVSLGDQASISCRSSQSLVHTNGNTYLVWYLPKPGSPK	50	(SEQ ID NO:99)
VK2-A18b germline(h)	1	DIVMTQTPLSLTVTPGQPASISCKSLIHSQKTYLVWYLPKPGSPQ	50	(SEQ ID NO:100)
4B10 Humanized LC	1	DIVMTQTPLSLTVTPGQPASISCRSSQSLVHTNGNTYLVWYLPKPGSPQ	50	(SEQ ID NO:101)
		*****.*		
		*****.*		
		*****.*		
4B10 light chain(m)	51	LLIYMVSNRFGVPDRFSGSGGTFTLKISRVEAEDIGIFVCSQSAHP	100	
VK2-A18b germline(h)	51	LLIYVSSRFSGVPDRFSGSGGTFTLKISRVEAEDVGVYCHQGIHLP	100	
4B10 Humanized LC	51	LLIYMVSNRFGVPDRFSGSGGTFTLKISRVEAEDVGVYCSQSAHP	100	
		*****.*		
		*****.*		
		*****.*		

FIG 16

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Humanized 4B10 kappa (light chain) variable region

DIYMTQTPLSLSVTPGQPASISCRSSQSLVHTNGNTYLHWYLQKPGQSPQLLIYMVSNRFSVPDRFSGSGGTDFTLKISRVEAEDVGVVYC
SQSAHVPPPLTFGQGTREIK
(SEQ ID NO:102)

Humanized 4B10 heavy chain variable region

EVOLVESGGGLVQPGGSLRLSCAASGFTPSDYMSWVRQAPGKLEWVGFI RNKANGYTTTEYSASVKGRFTISRDDSKNSLYLQMNLSLKTEDT
AVYFCARYIWFDYWGQGTITVYS
(SEQ ID NO:103)

FIG 17

FIG. 18

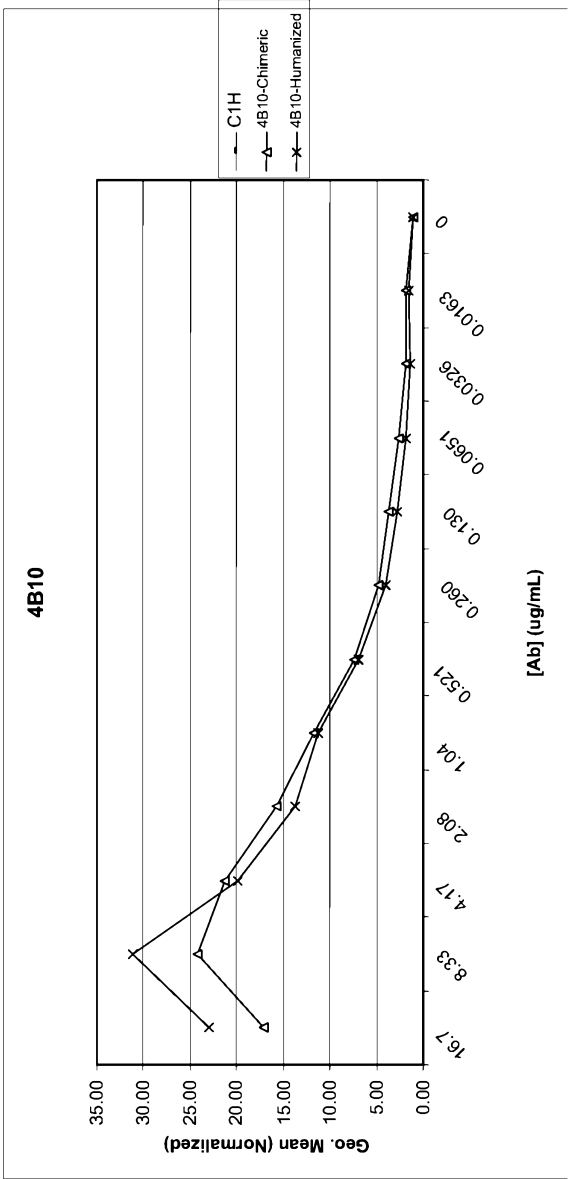
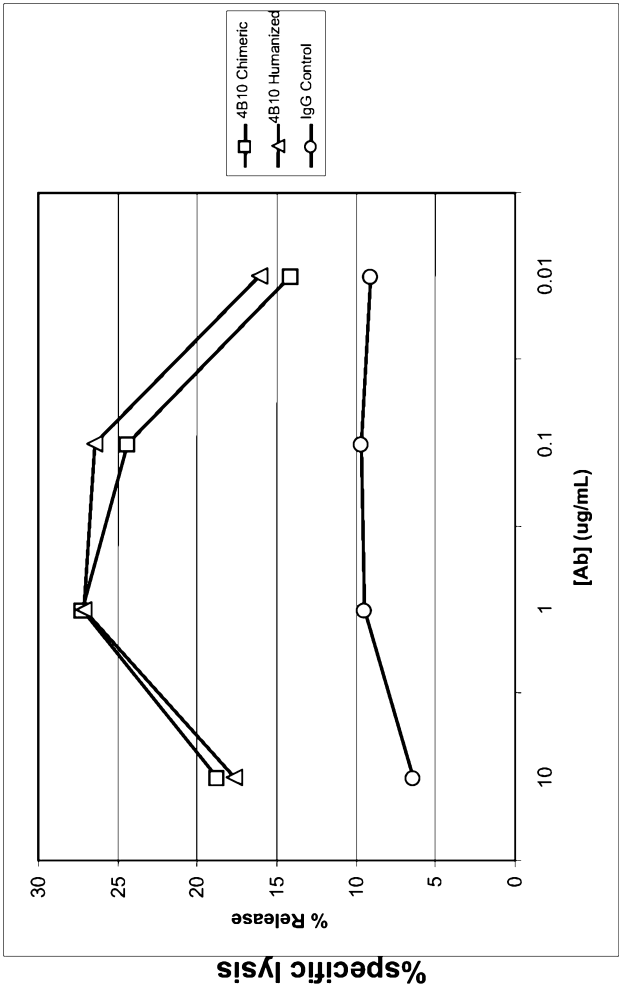


FIG. 19



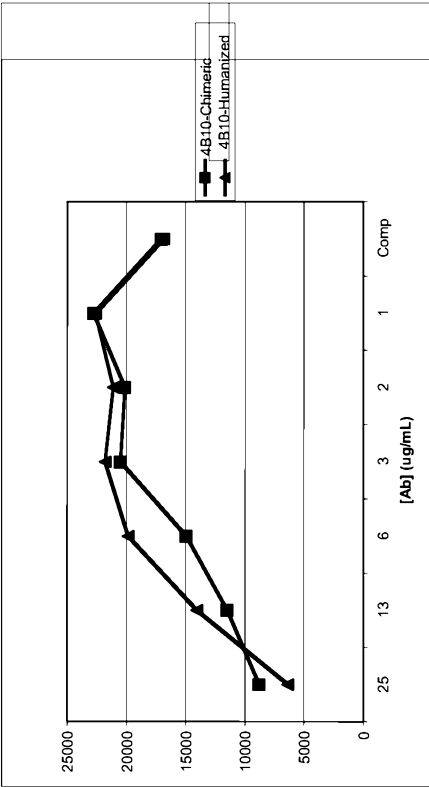
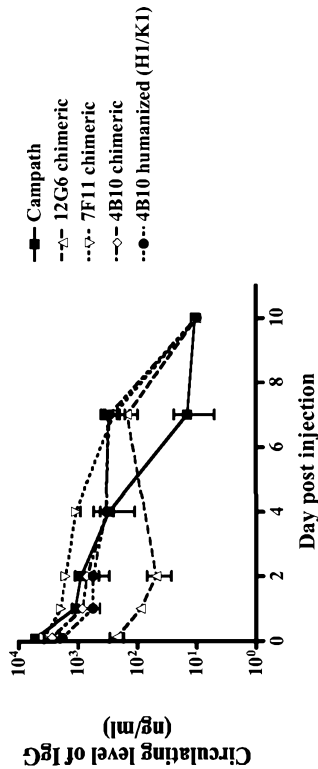
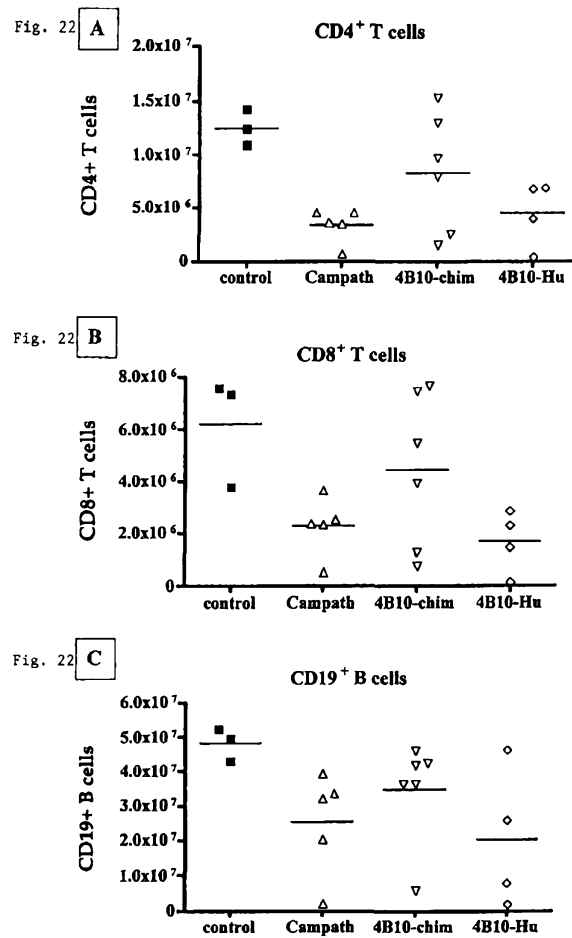


FIG.20

FIG. 21



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**FIG. 22A - 22C**

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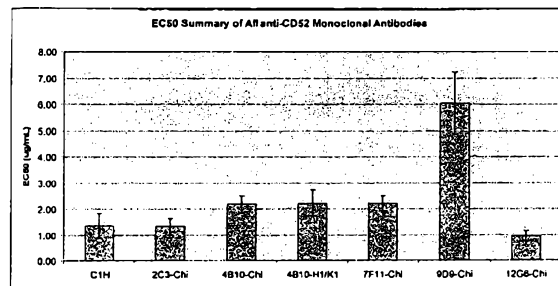


FIG. 23

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Humanized 7F11 clone heavy chain variable region sequences

7F11-SFD1

EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYMSWVRQAPGKGLEWVGFIRNK
ANGYTTEYNASVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCTRYIFFDYWG
QGTTVTVSS (SEQ ID NO: 136)

7F11-SFD2

EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYMSWVRQAPGKGLEWVGFIRNK
ANGYTTEYNASVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCARYIFFDYWG
QGTTVTVSS (SEQ ID NO: 137)

Humanized 7F11 clone light (kappa) chain variable region sequence

7F11-VK2

DIVMTQTPLSLSVTPGQPASISCRSSQSLVHTNGNSYLHWYLQKPGQSPQLLIYMV
SNRFGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSTHVPFTFGQGTKLEIK
(SEQ ID NO: 138)

FIG. 24

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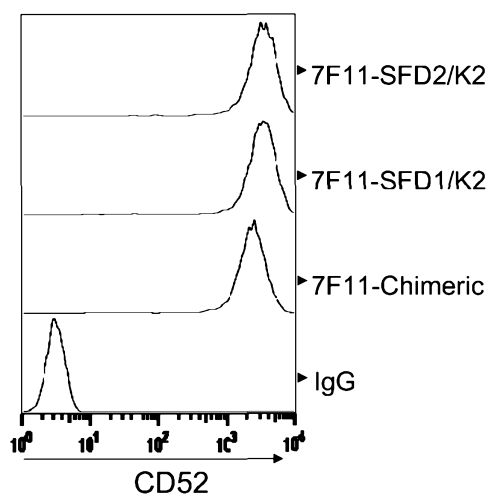


FIG. 25

Humanized 2C3 clone heavy chain variable region sequences

2C3-SFD1
EVQLVESGGGLVQPGGSLRLSCAASGFTFTNTYWMNWRQAPGKGLEWVGQIRLKSNNYATHYAESVKGRFTISRDDSKNSLYLQM
NSLKTEDTAVYYCTPVD^{FW}GQTTTVSS (SEQ ID NO: 139)

2C3-VH 12
EVQLVESGGGLVQPGGSLRLSCAASGFTFTNTYWMNWRQAPGKGLEWVGQIRLKSNNYATHYAESVKGRFTISRDDSKNSVYLQ
MNSLKTEDTAVYYCTPVD^{FW}GQTTTVSS (SEQ ID NO: 140)

2C3-VH 15
EVQLVESGGGLVQPGGSLRLSCAASGFTFTNTYWMNWRQAPGKGLEWV^AQIRLKSNNYATHYAESVKGRFTISRDDSKNSLYLQM
NSLKTEDTAVYYCTPVD^{FW}GQTTTVSS (SEQ ID NO: 141)

2C3-VH16
EVQLVESGGGLVQPGGSLRLSCAASGFTFTNTYWMNWRQAPGKGLEWV^AQIRLKSNNYATHYAESVKGRFTISRDDSKNSVYLQ
MNSLKTEDTAVYYCTPVD^{FW}GQTTTVSS (SEQ ID NO: 142)

2C3-VH17
EVQLVESGGGLVQPGGSMRLSCAASGFTFTNTYWMNWRQAPGKGLEWV^AQIRLKSNNYATHYAESVKGRFTISRDDSKNSVYLQ
MNSLKTEDTAVYYCTPVD^{FW}GQTTTVSS (SEQ ID NO: 143)

2C3-VH19
EVQLVESGGGLVQPGGSMRLSCAASGFTFTNTYWMNWRQAP^EKGLEWV^AQIRLKSNNYATHYAESVKGRFTISRDDSKNSVYLQ
MNSLKTEDTAVYYCTPVD^{FW}GQTTTVSS (SEQ ID NO: 144)

FIG. 26A

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Humanized 2C3 clone light (kappa) chain variable region sequences**2C3-VK1**

DI¹VM¹TQ¹TPL¹SL¹SVTPGQ¹PASISCK¹SSQ¹SL¹YSNGK¹TYL¹NWYL¹LQ¹PKGQSPQ¹RLI¹YLV¹SK¹LD¹SGV¹PDFR¹SGSG¹SGTD¹FTL¹KISR¹VEAE
DVG¹VYYCV¹QG¹THL¹HT¹FGQ¹TRLEIK (SEQ ID NO: 145)

2C3-VK11

DI¹VM¹TQ¹TPL¹SL¹SVTPGQ¹PASISCK¹SSQ¹SL¹YSNGK¹TYL¹NWYL¹LQ¹PKGQSPQ¹RLI¹YLV¹SK¹LD¹SGV¹PDFR¹SGSG¹SGTD¹FTL¹KISR¹VEAE
DVG¹VYYCV¹QG¹THL¹HT¹FGQ¹TRLEIK (SEQ ID NO: 146)

2C3-VK12

DI¹VM¹TQ¹TPL¹SL¹SVTPGQ¹PASISCK¹SSQ¹SL¹YSNGK¹TYL¹NWLL¹LQ¹PKGQSPQ¹RLI¹YLV¹SK¹LD¹SGV¹PDFR¹SGSG¹SGTD¹FTL¹KISR¹VEAE
DVG¹VYYCV¹QG¹THL¹HT¹FGQ¹TRLEIK (SEQ ID NO: 147)

2C3-VK13

DI¹VT¹TQ¹TPL¹SL¹SVTPGQ¹PYSISCK¹SSQ¹SL¹YSNGK¹TYL¹NWLL¹LQ¹PKGQSP¹KRLI¹YLV¹SK¹LD¹SGV¹PDFR¹SGSG¹SGTD¹FTL¹KISR¹VEAE
DVG¹VYYCV¹QG¹THL¹HT¹FGQ¹TRLEIK (SEQ ID NO: 148)

FIG. 26B

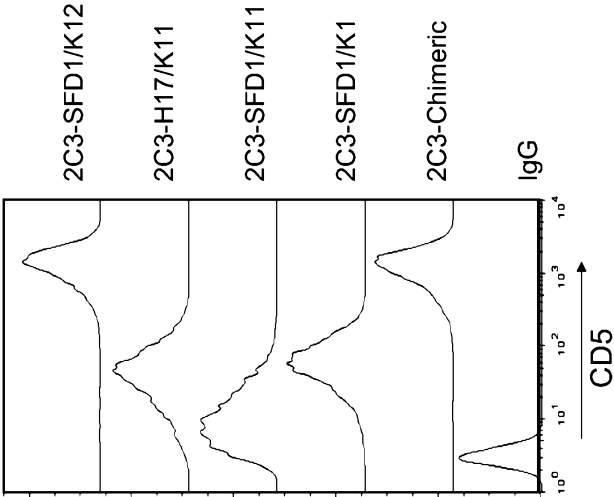


FIG. 27A

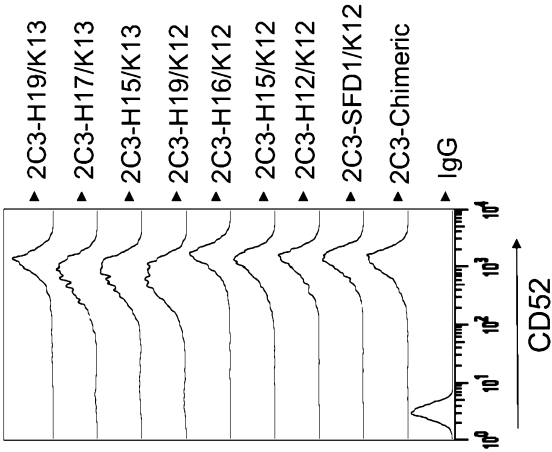


FIG. 27B

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Humanized 12G6 clone heavy chain variable region sequences**12G6-SFD1**

EVQLVESGGGLVQPGGSLRLSCAASGFPFSNYWMNWVRQAPGKGLEWVGQIRLK
SNNYATHYAESVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCTPIDYWGQGTT
VTVSS (SEQ ID NO: 149)

12G6-VH 10

EVQLVESGGGLVQPGGSLRLSCAASGFPFSNYWMNWVRQAPGKGLEWVGQIRLK
SNNYATHYAESVKGRFTISRDDSKNSVYLQMNSLKTEDTAVYYCTPIDYWGQGTT
VTVSS (SEQ ID NO: 150)

12G6-VH 11

EVQLVESGGGLVQPGGSLRLSCAASGFPFSNYWMNWVRQAPGKGLEWVAQIRLK
SNNYATHYAESVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCTPIDYWGQGTT
VTVSS (SEQ ID NO: 151)

12G6-VH12

EVQLVESGGGLVQPGGSLRLSCAASGFPFSNYWMNWVRQAPGKGLEWVAQIRLK
SNNYATHYAESVKGRFTISRDDSKNSVYLQMNSLKTEDTAVYYCTPIDYWGQGTT
VTVSS (SEQ ID NO: 152)

FIG. 28A

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Humanized 12G6 clone light (kappa) chain variable region sequence**12G6-VK1**

DIVMTQTPLSLSVTPGQPASISCKSSQSLLYSNGKTYLNWYLQKPGQSPQLLIY
LVSKLDSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGSHFHTFGQGGTK
LEIK (SEQ ID NO: 153)

12G6-VK10

DIVMTQTPLSLSVTPGQPASISCKSSQSLLYSNGKTYLNWYLQKPGQSPQLLIY
LVSKLDSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGSHFHTFGQGGTK
LEIK (SEQ ID NO: 154)

12G6-VK11

DIVMTQTPLSLSVTPGQPASISCKSSQSLLYSNGKTYLNWYLQKPGQSPKRLIY
LVSKLDSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGSHFHTFGQGGTK
LEIK (SEQ ID NO: 155)

12G6-VK12

DIVMTQTPLSLSVTPGQPASISCKSSQSLLYSNGKTYLNWYLQKPGQSPQRLIY
LVSKLDSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGSHFHTFGQGGTK
LEIK (SEQ ID NO: 156)

12G6-VK13

DIVMTQTPLSLSVTPGQPASISCKSSQSLLYSNGKTYLNWYLQKPGQSPQRLIY
LVSKLDSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGSHFHTFGQGGTK
LEIK (SEQ ID NO: 157)

FIG. 28B

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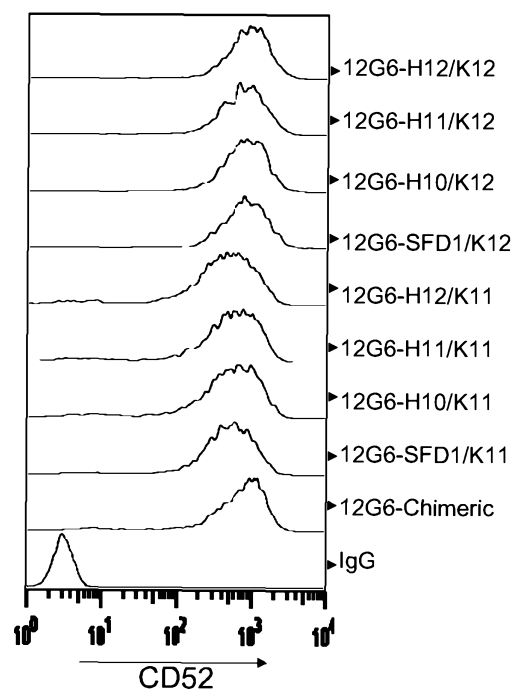


FIG. 29

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Humanized 9D9 clone heavy chain variable region sequences**9D9-VH10**

EVQLLESGGGLVQPGGSLRLSCAASGFTFSDAWMDWVRQAPGKGLEWVSEIRNKA
AKNHATYYAESVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTTLD SWGQGT TVSS (SEQ ID NO: 158)

9D9-VH11

EVQLLESGGGLVQPGGSLRLSCAASGFTFSDAWMDWVRQAPGKGLELTAEIRNK
AKNHATYYAESVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTTLD SWGQGT TVTVSS (SEQ ID NO: 159)

9D9-VH15

EVQLLESGGGLVQPGGSLRLSCAASGFTFSDAWMDWVRQAPGKGLELTAEIRNK
AKNHATYYAESVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTTLD SWGQGT TVTVSS (SEQ ID NO: 160)

9D9-VH 16

EVQLLESGGGLVQPGGSLRLSCAASGFTFSDAWMDWVRQAPGKGLELVSEIRNK
AKNHATYYAESVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTTLD SWGQGT TVTVSS (SEQ ID NO: 161)

9D9-VH 17

EVQLLESGGGLVQPGGSLRLSCAASGFTFSDAWMDWVRQAPGKGLELTSEIRNK
AKNHATYYAESVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTTLD SWGQGT TVTVSS (SEQ ID NO: 162)

9D9-VH 18

EVQLLESGGGLVQPGGSLRLSCAASGFTFSDAWMDWVRQAPGKGLELVAEIRNK
AKNHATYYAESVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTTLD SWGQGT TVTVSS (SEQ ID NO: 163)

FIG. 30A

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Humanized 9D9 clone light (kappa) chain variable region sequences**9D9-VK2**

DIVMTQTPLSLSVTPGQPASISCKSSQSLDSDGKTYLNWYLQKPGQSPQLLIYL
VSALDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCWQGTHFPWTFGQGTK
LEIK (SEQ ID NO: 164)

9D9-VK12

DIVMTQTPLSLSVTPGQPASISCKSSQSLDSDGKTYLNWLLQKPGQSPKRLLIYL
VSALDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCWQGTHFPWTFGQGTK
LEIK (SEQ ID NO: 165)

9D9-VK13

DIVMTQTPLSLSVTPGQPASISCKSSQSLDSDGKTYLNWLLQKPGQSPQRLLIYL
VSALDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCWQGTHFPWTFGQGTK
LEIK (SEQ ID NO: 166)

9D9-VK14

DIVMTQTPLSLSVTPGQPASISCKSSQSLDSDGKTYLNWYLQKPGQSPQRLLIYL
VSALDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCWQGTHFPWTFGQGTK
LEIK (SEQ ID NO: 167)

9D9-VK15

DIVMTQTPLSLSVTPGQPASISCKSSQSLDSDGKTYLNWYLQKPGQSPKRLLIYL
VSALDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCWQGTHFPWTFGQGTK
LEIK (SEQ ID NO: 168)

FIG. 30B

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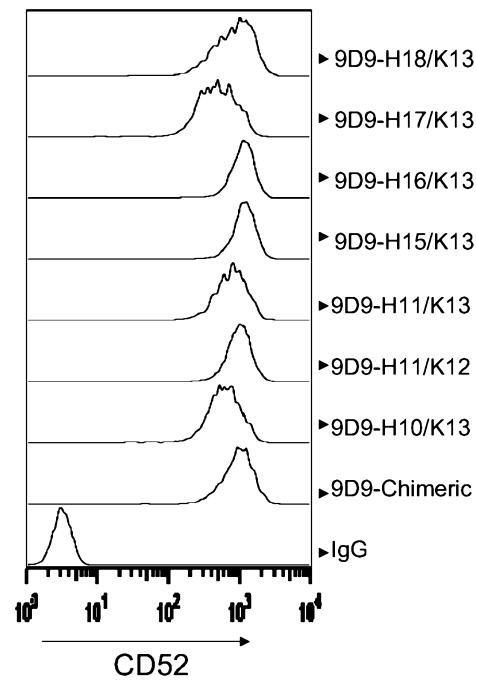


FIG. 31

Human CD4+ T cells hCD52 Tg mouse CD4+ T

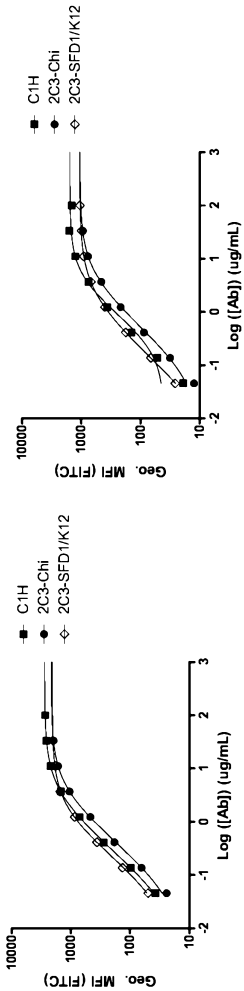


FIG. 32A

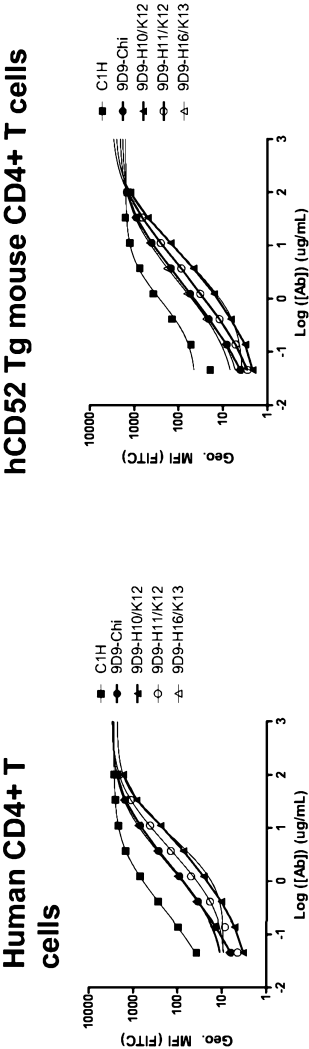


FIG. 32B

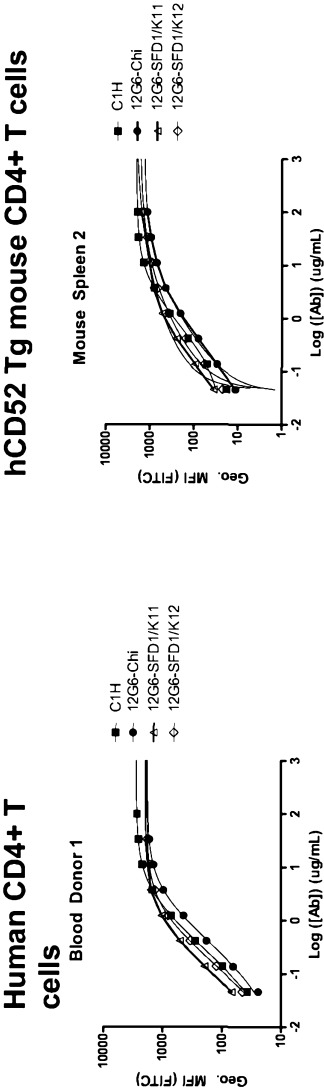


FIG. 32C

	EC ₅₀ (nM)	
	Human CD4+ T cells	huCD52 transgenic mouse CD4+ T cells
Campath-1H®	21.91	25.08
2C3-Chimeric	25.93	30.81
2C3-SFD1/K12	11.00	13.61
12G6-Chimeric	22.41	34.42
12G6-SFD1/K11	7.30	13.17
12G6-SFD1/K12	10.58	

FIG. 33

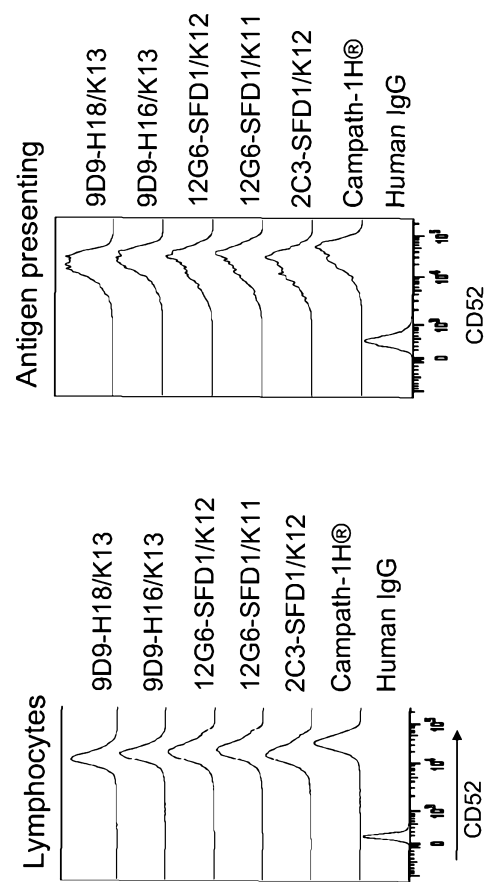


FIG. 34

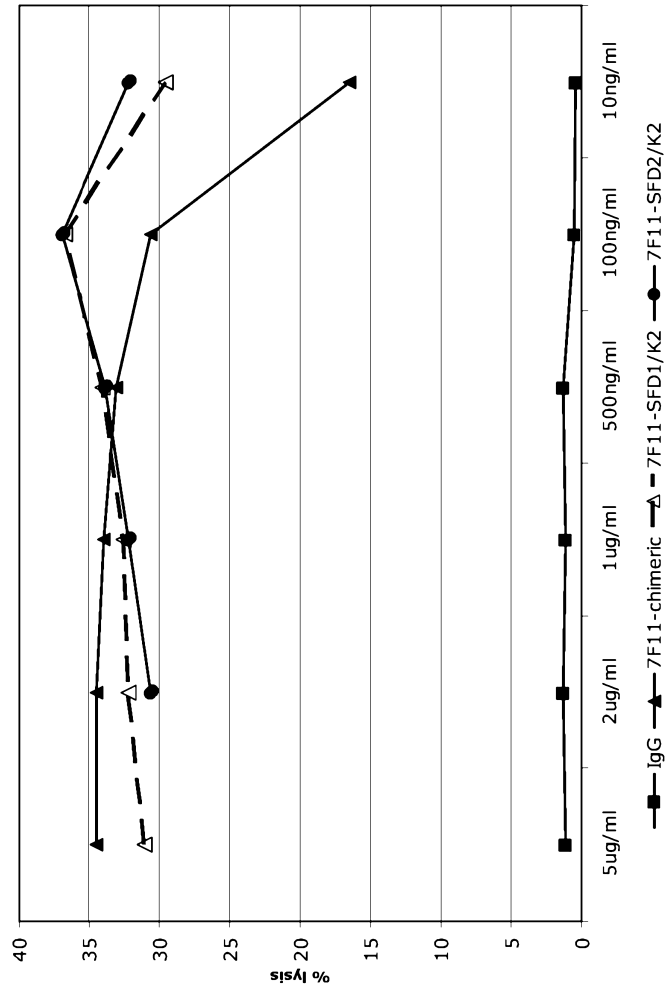


FIG. 35

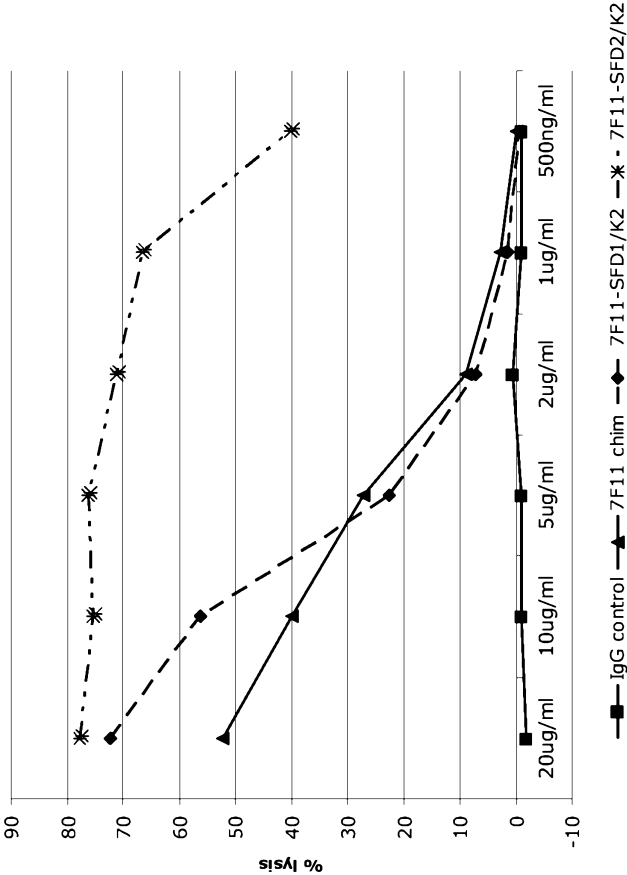


FIG. 36

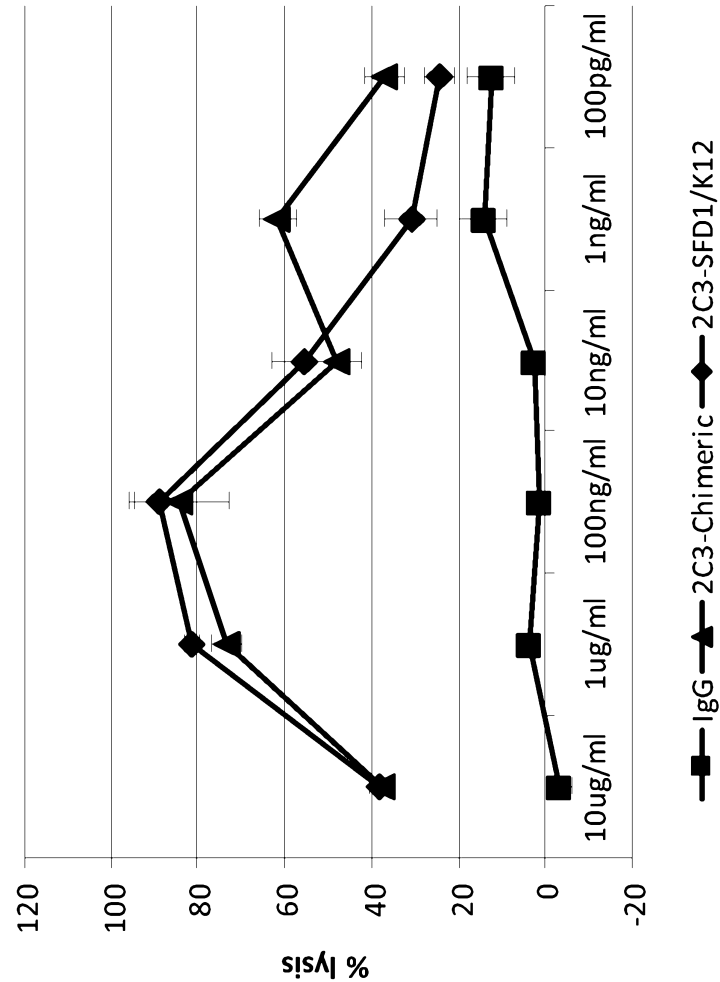


FIG. 37

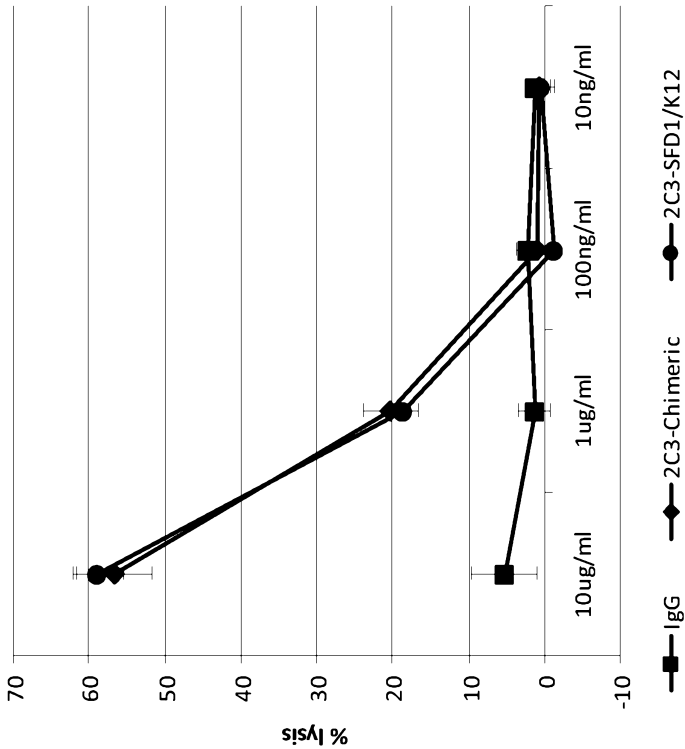


FIG. 38

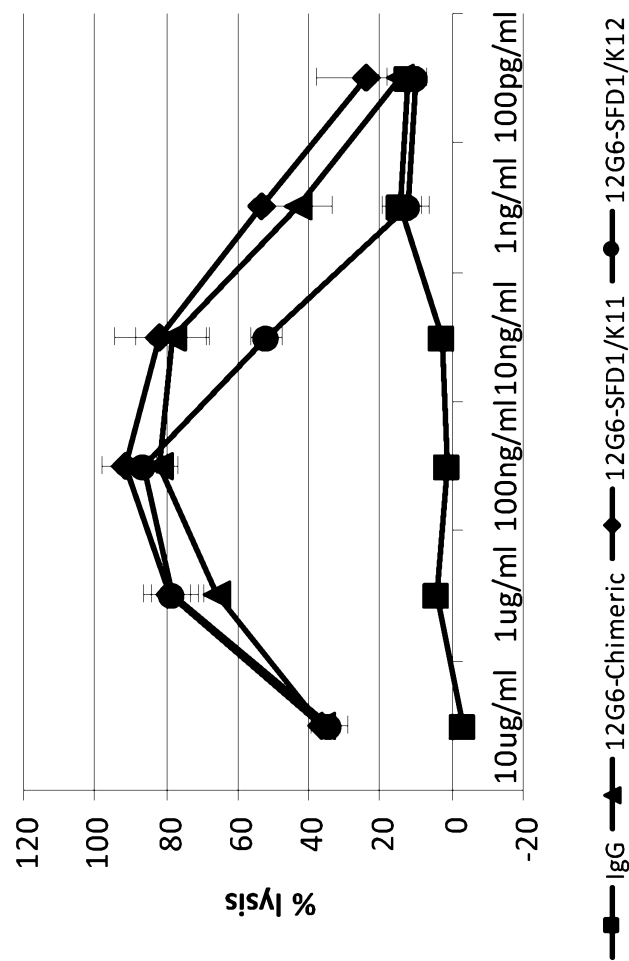


FIG. 39

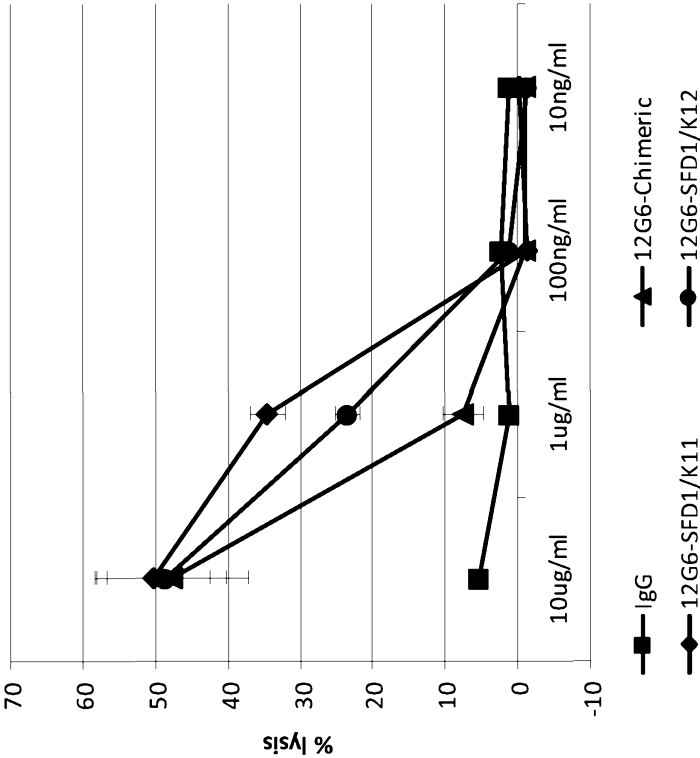
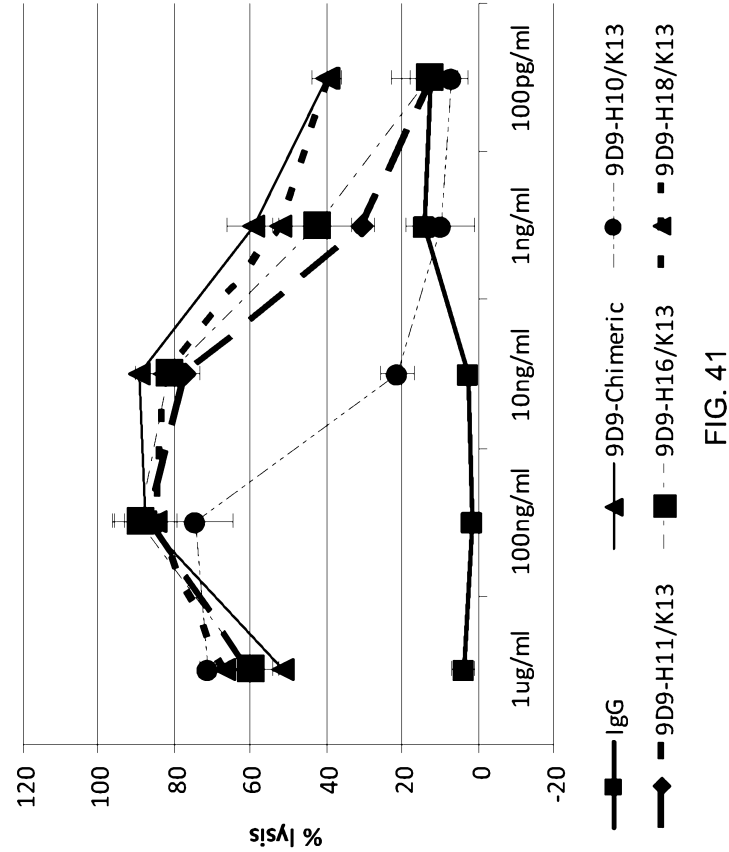


FIG. 40



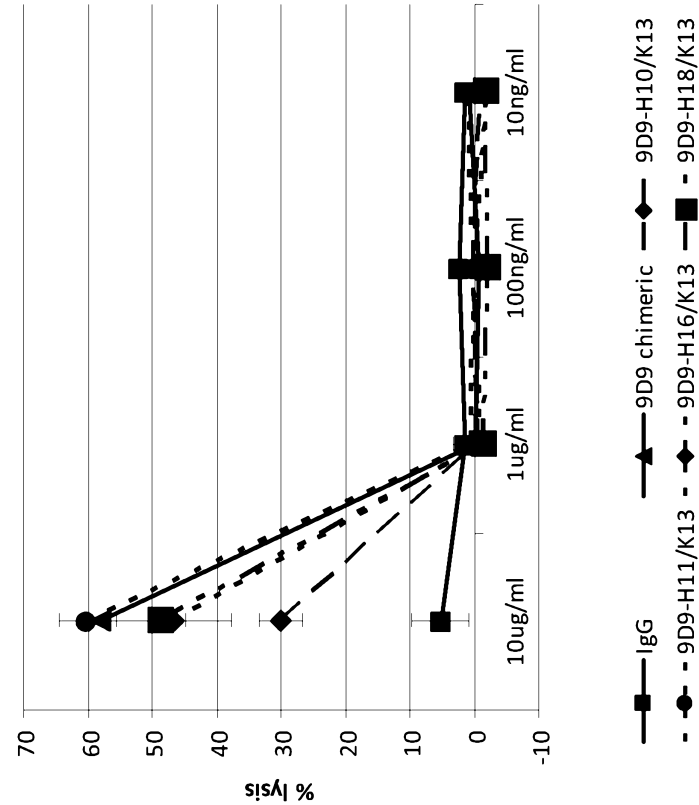


FIG. 42

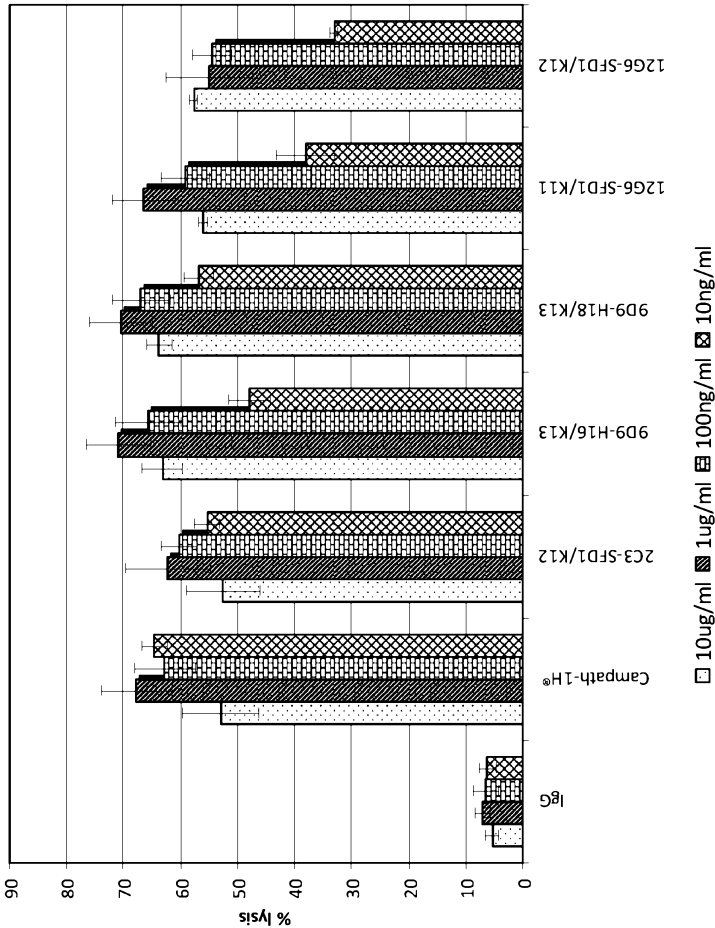


FIG. 43

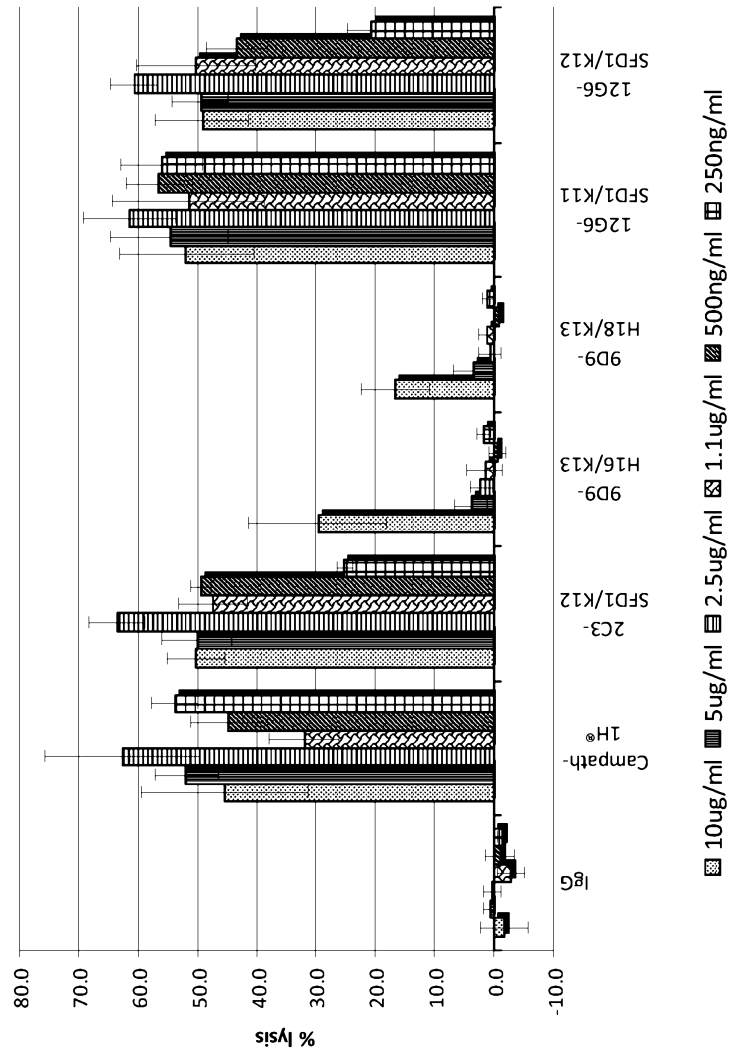


FIG. 44

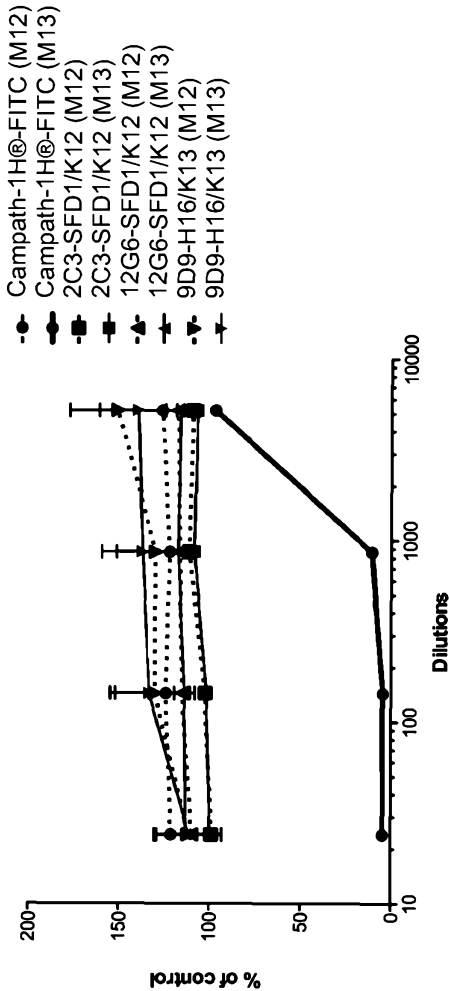


FIG. 45

FIG. 46A

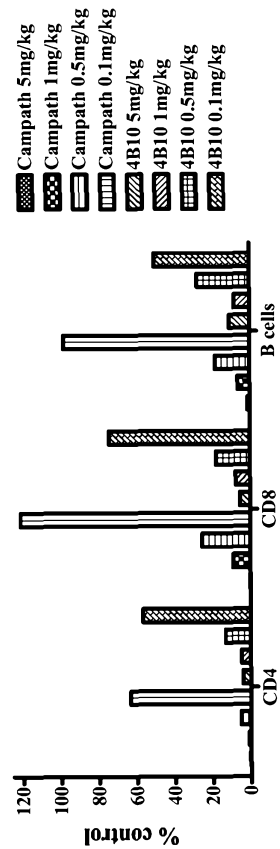
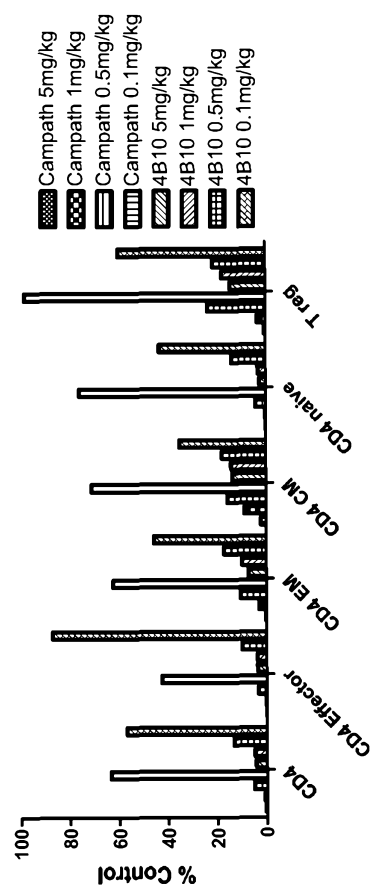


FIG. 46B



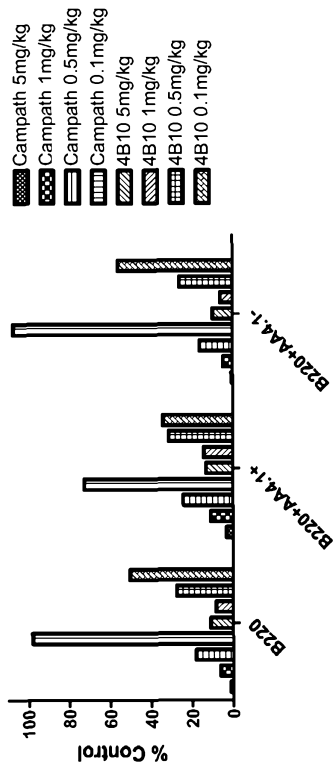


FIG. 46D

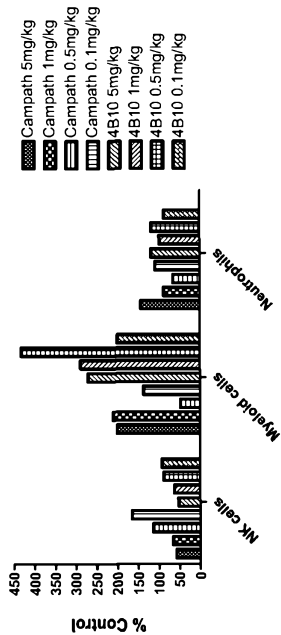
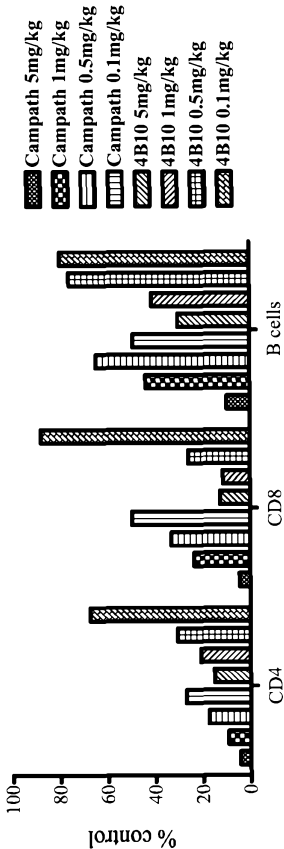


FIG. 46E

FIG. 47A



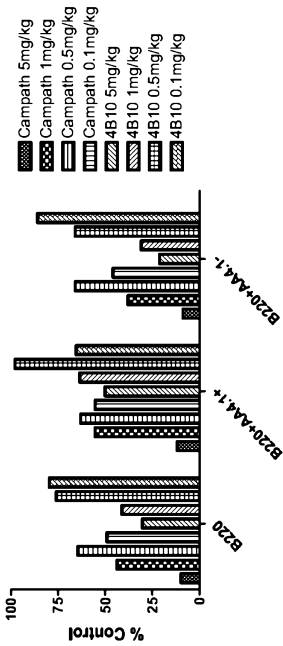


FIG. 47D

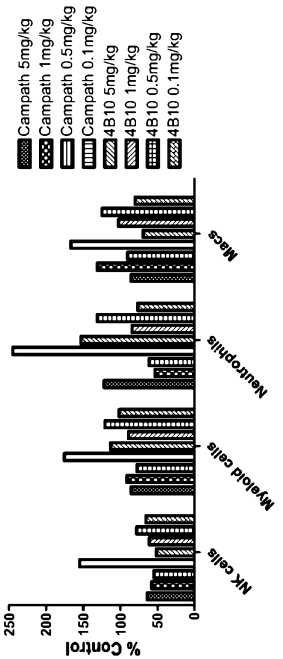


FIG. 47E

FIG. 48

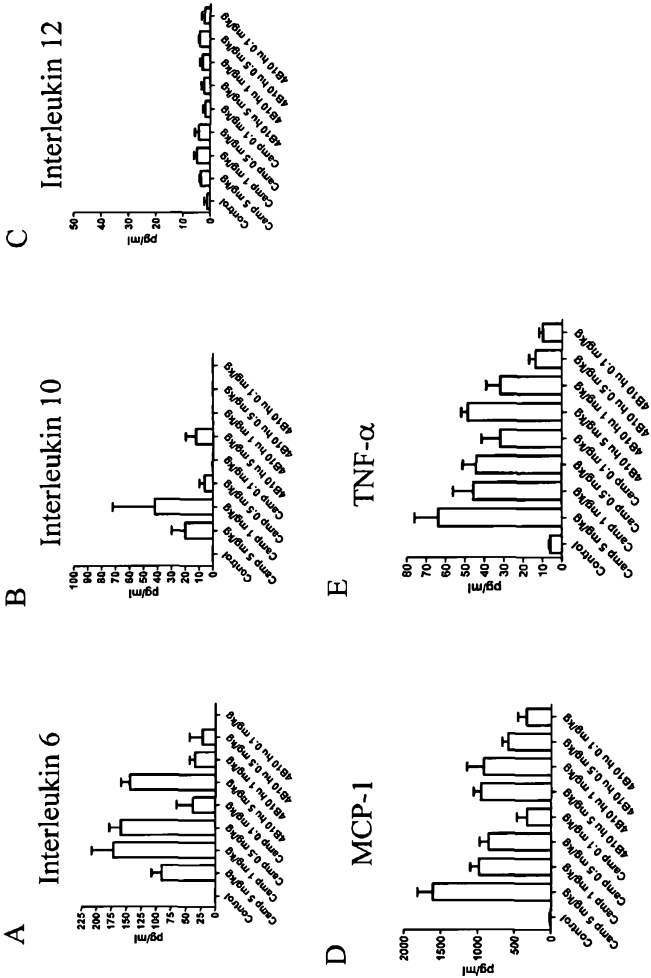


FIG. 49

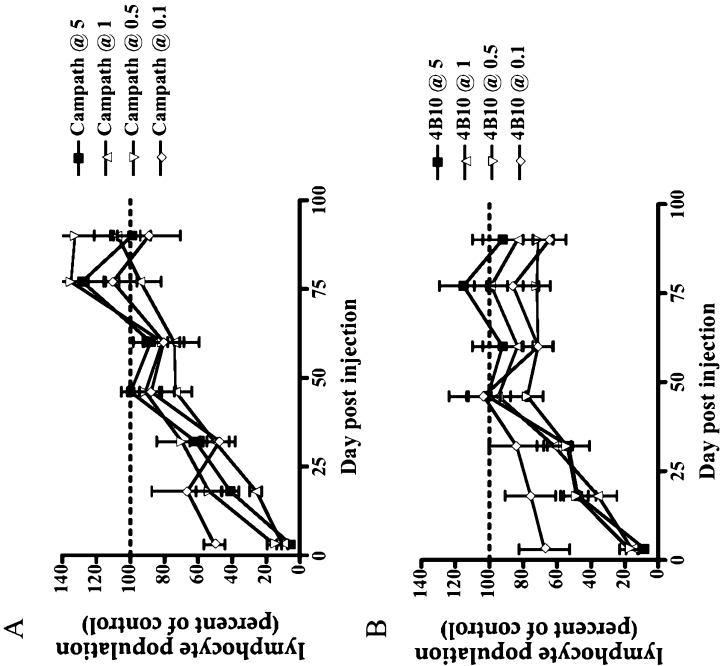
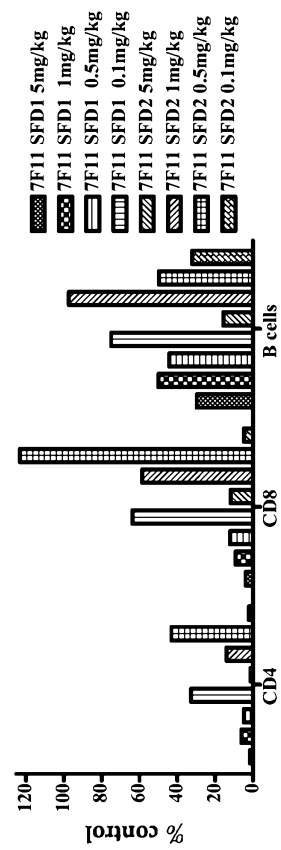


FIG. 50A



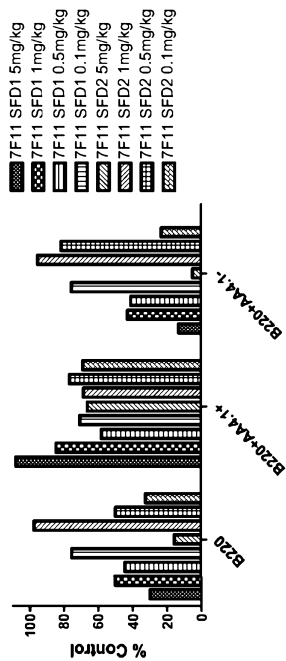


FIG. 50D

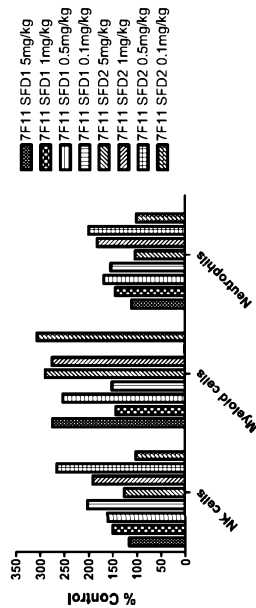
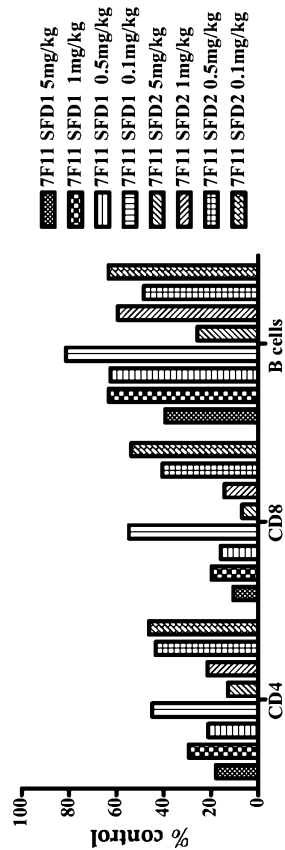


FIG. 50E

FIG. 51A



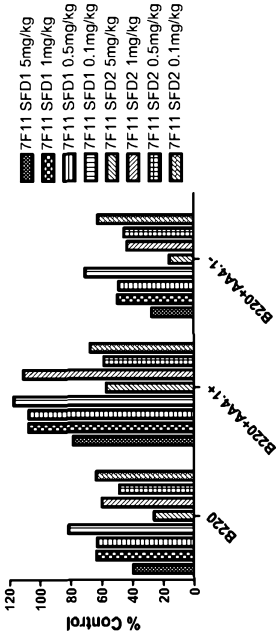


FIG. 51D

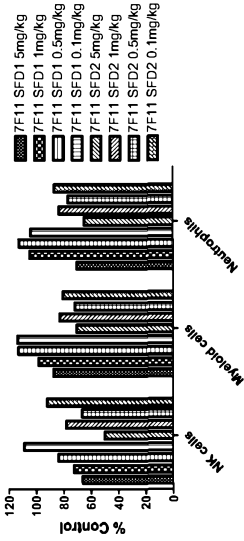


FIG. 51E

FIG. 52

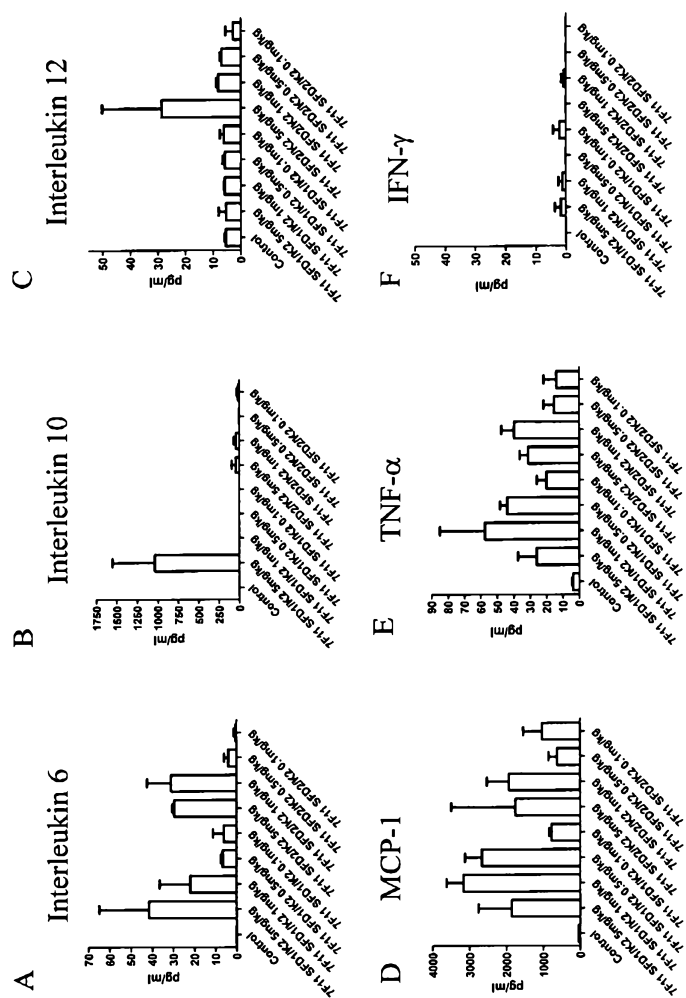


FIG. 53

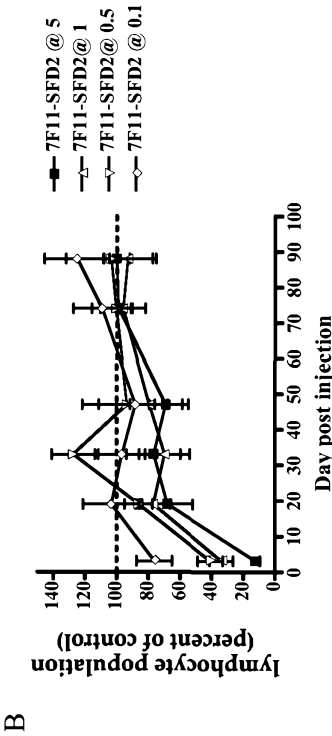
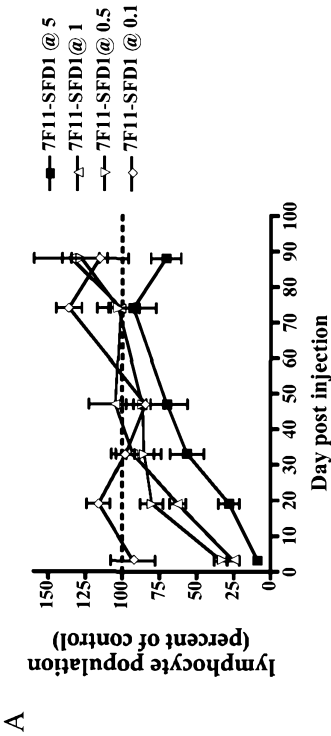
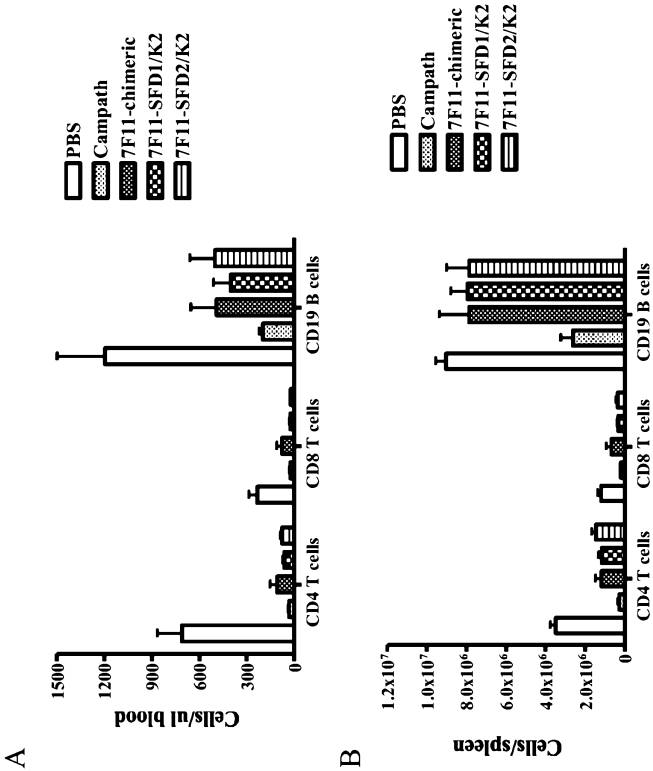


FIG. 54



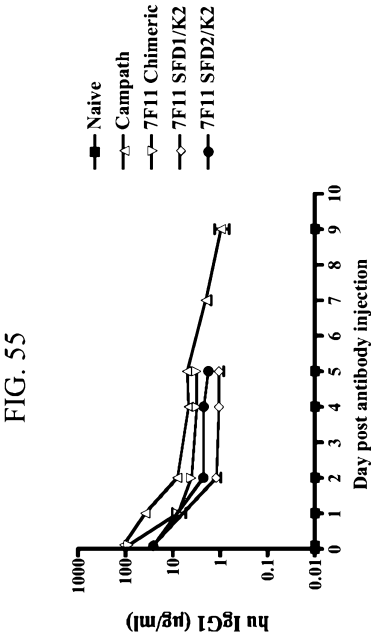


FIG. 56A

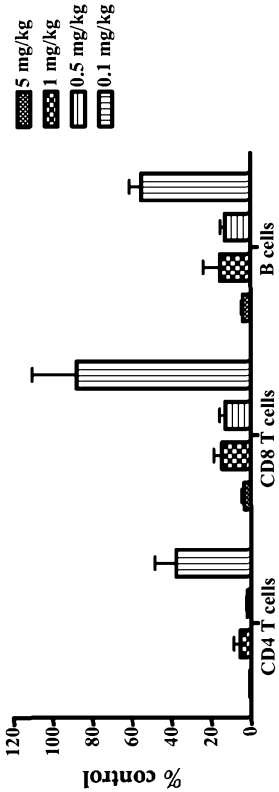


FIG. 56B

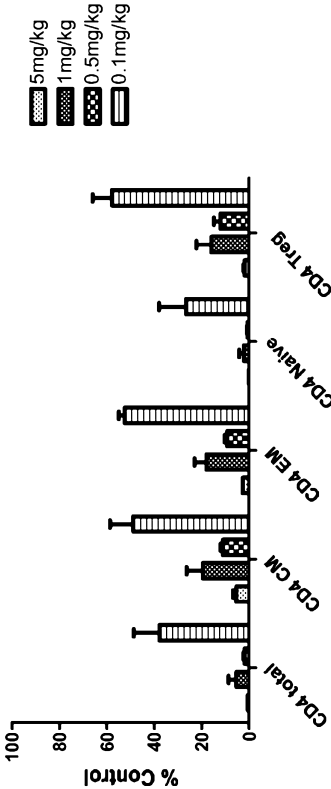
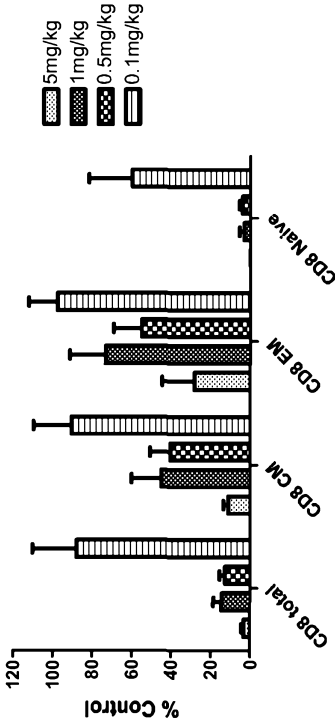


FIG. 56C



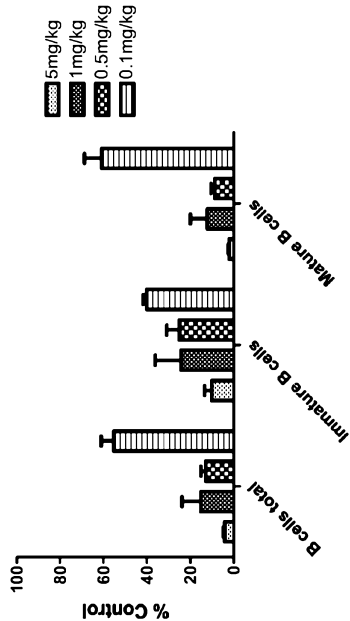


FIG. 56D

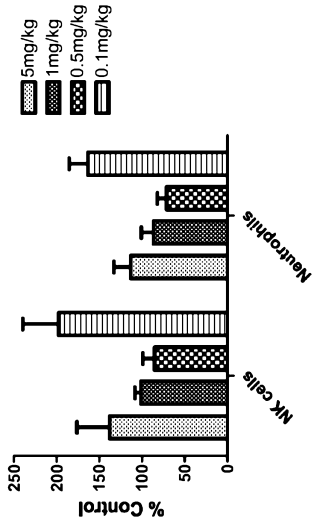


FIG. 56E

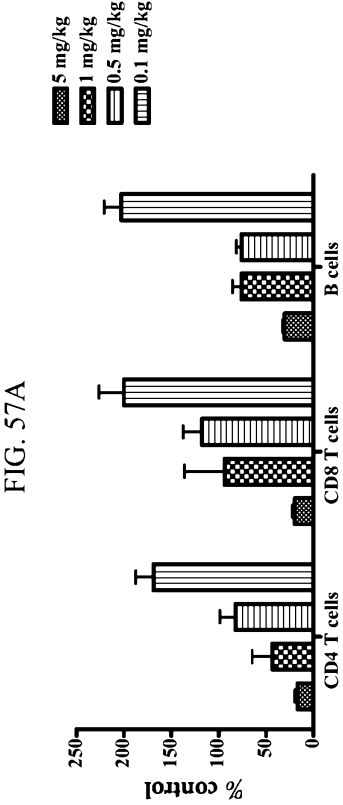


FIG. 57B

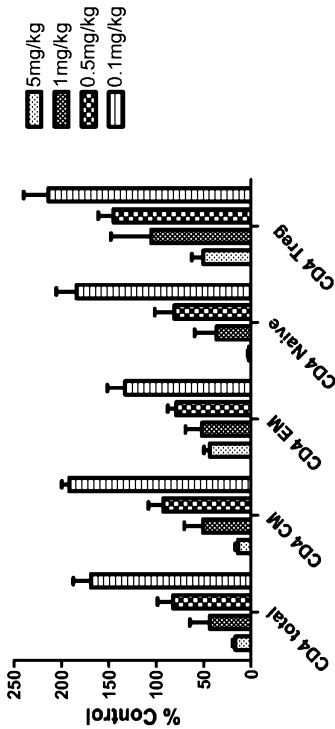
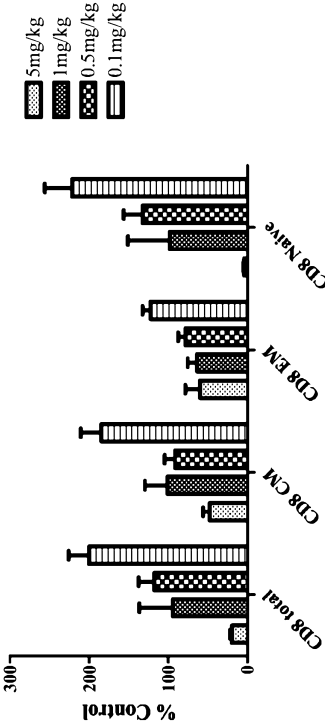


FIG. 57C



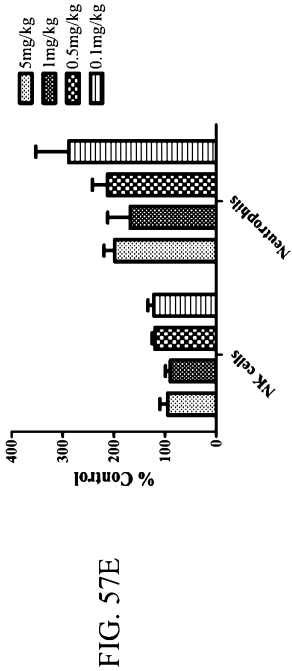
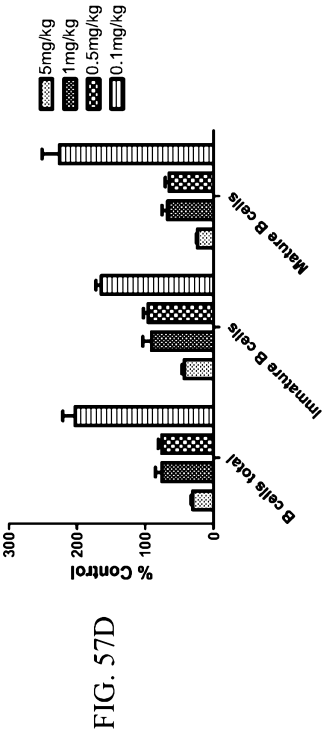


FIG. 58

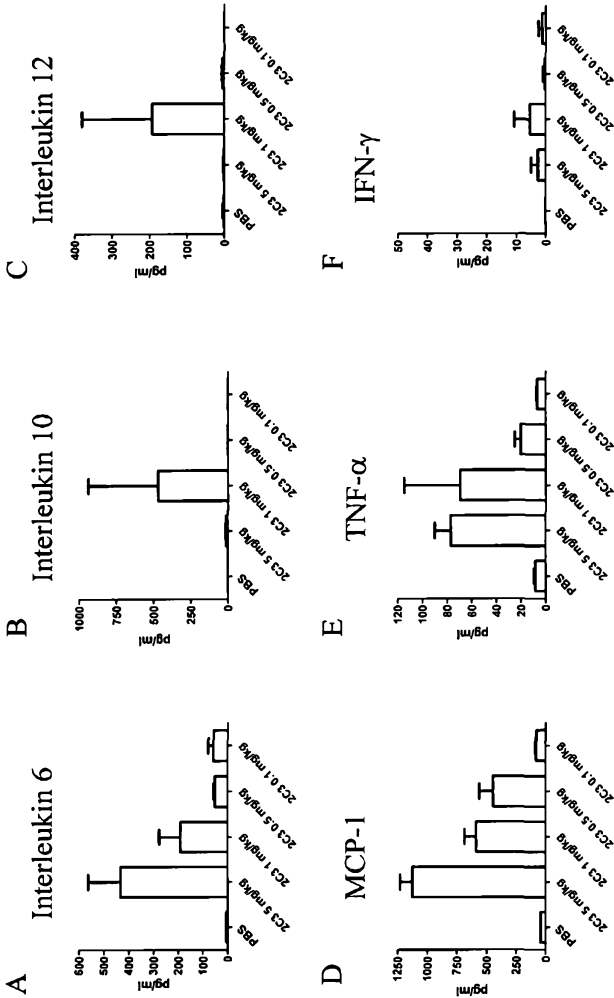


FIG. 59

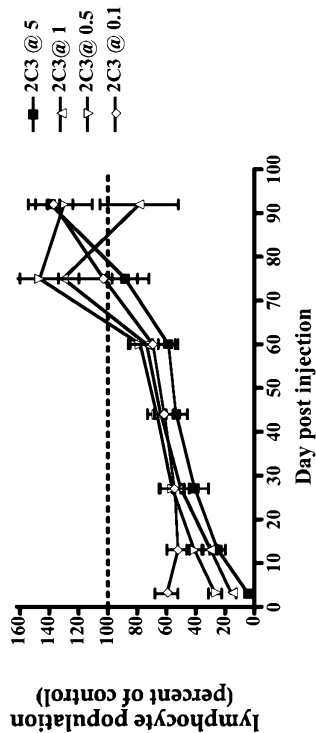
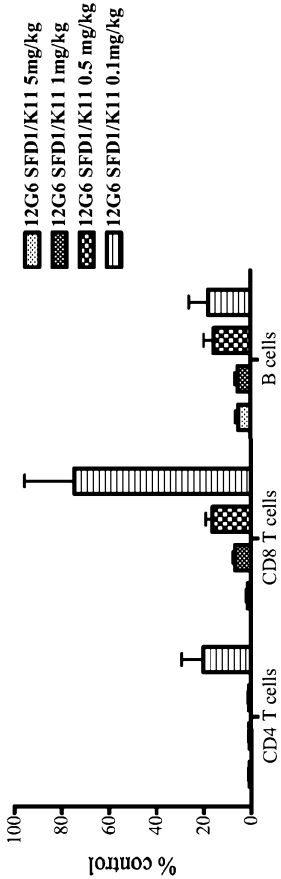


FIG. 60A



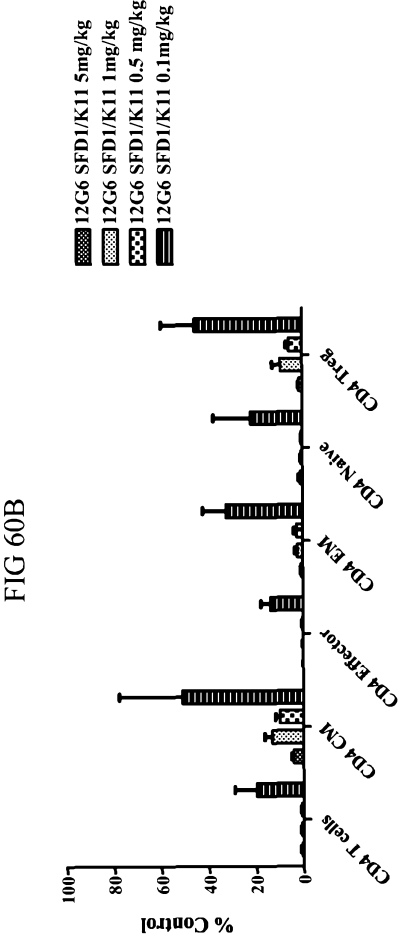
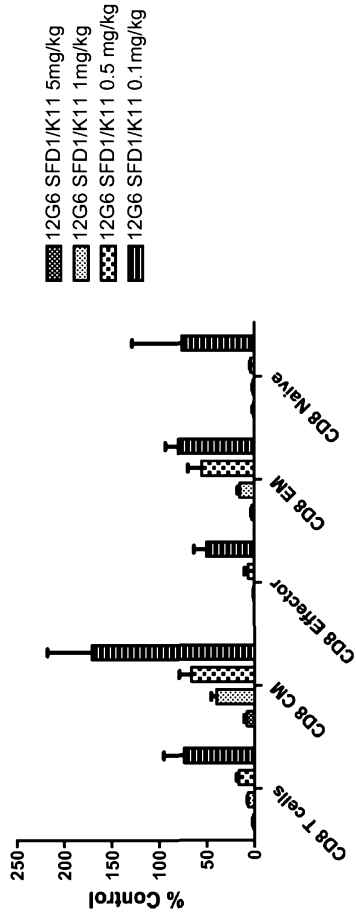


FIG. 60C



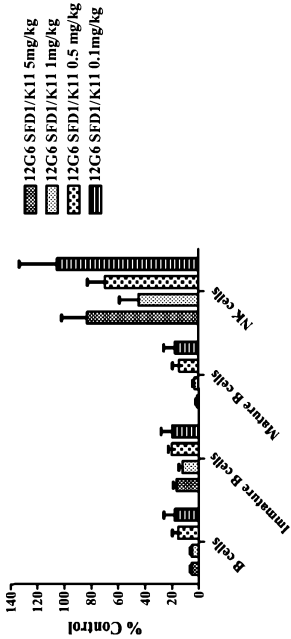


FIG. 60D

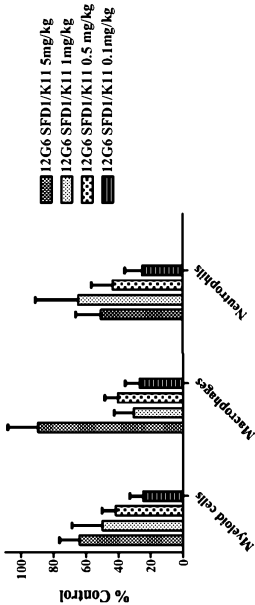


FIG. 60E

FIG. 61A

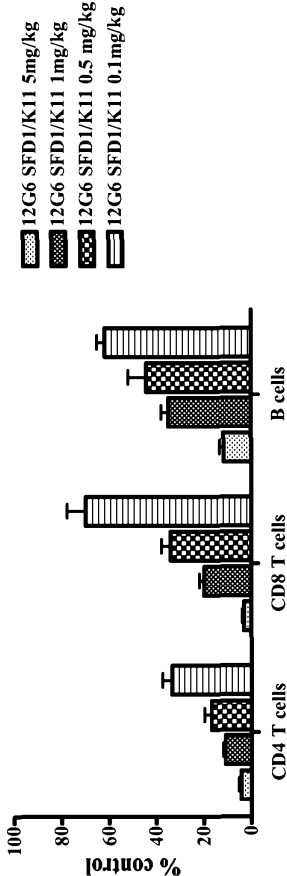


FIG. 61B

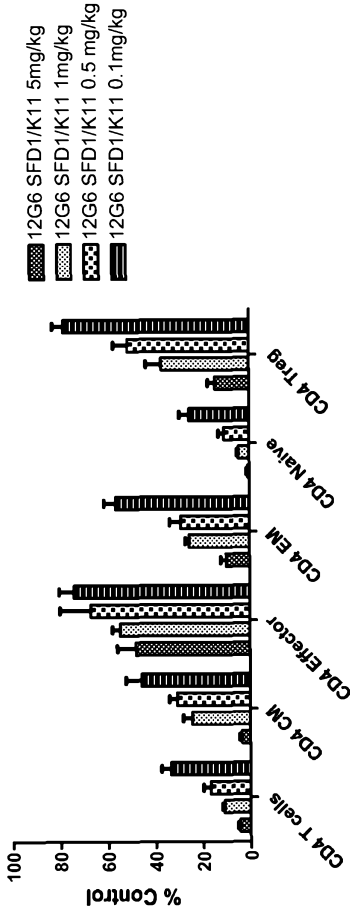
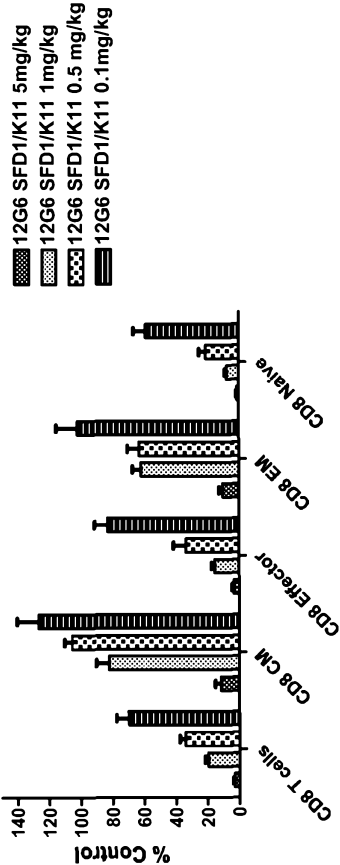


FIG. 61C



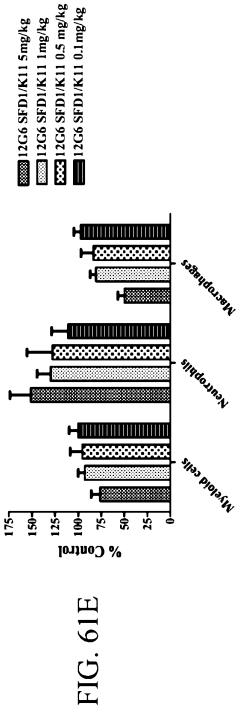
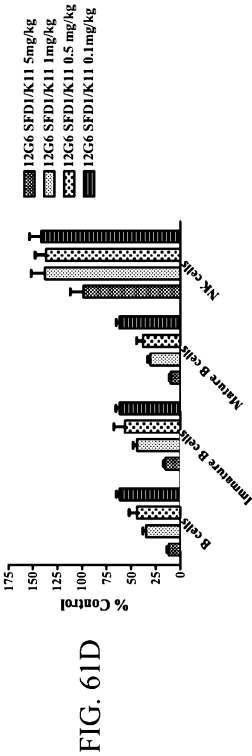


FIG. 62

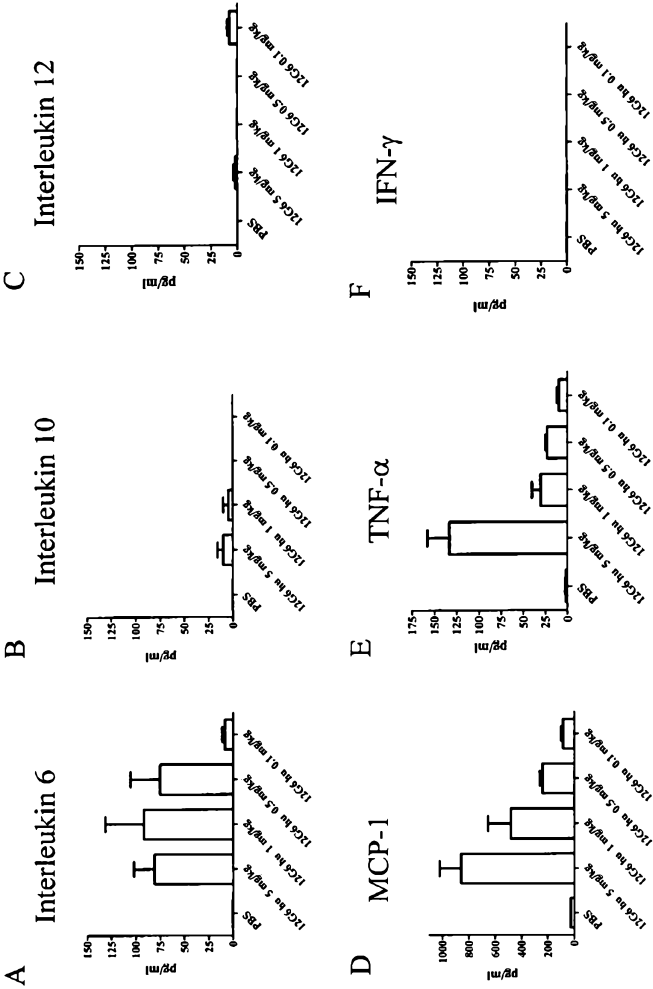


FIG. 63

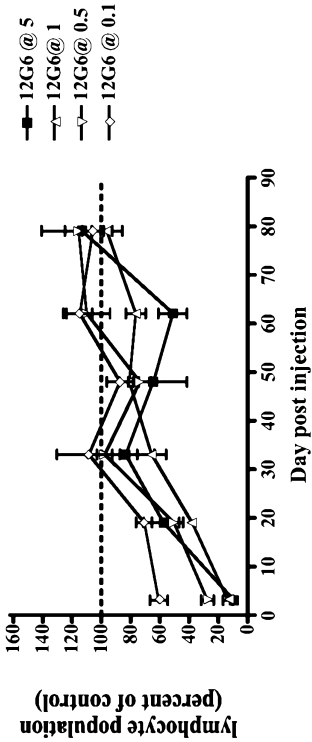


FIG. 64

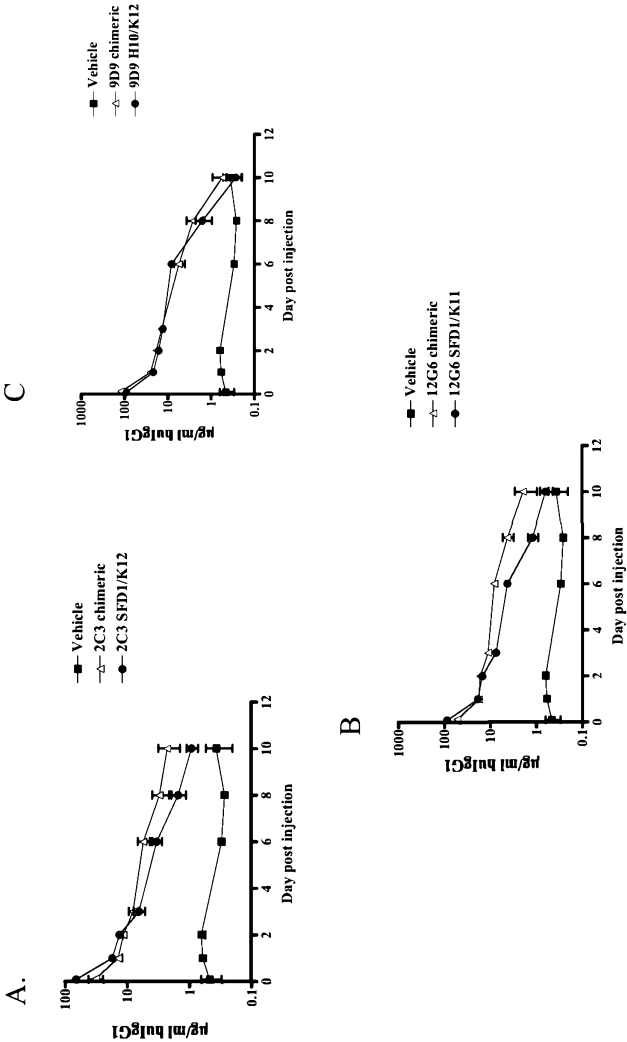
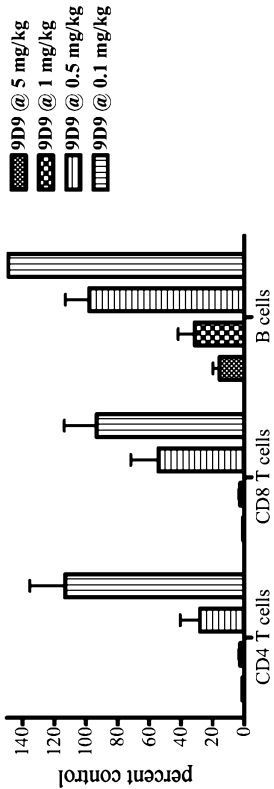


FIG. 65A



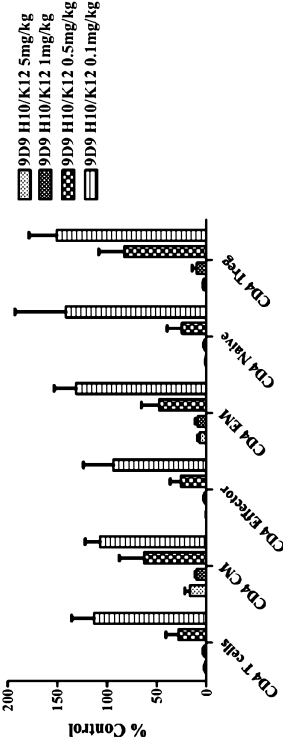


FIG. 65B

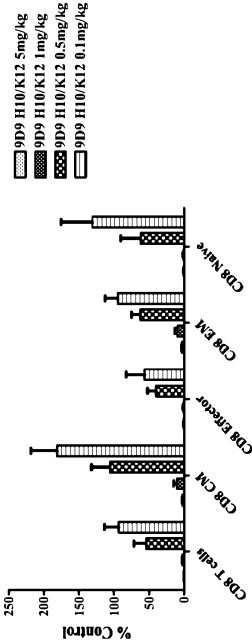
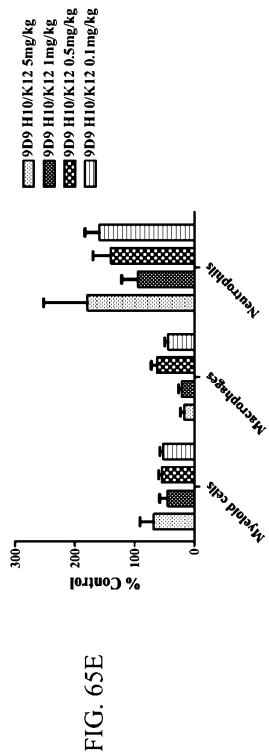
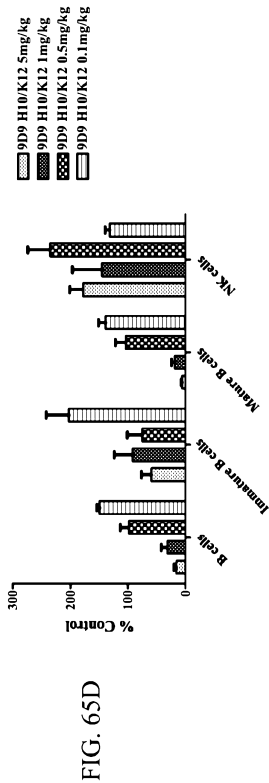
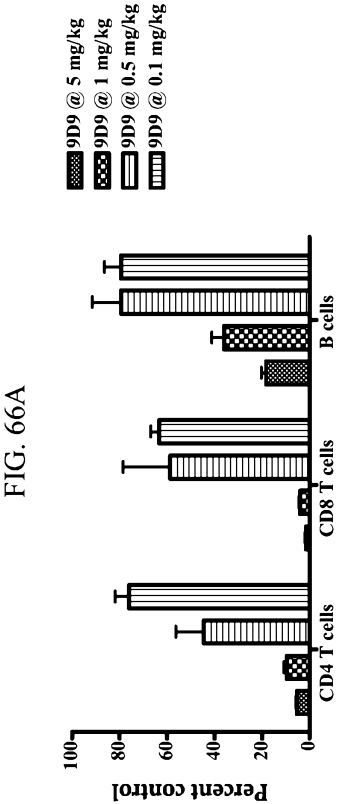
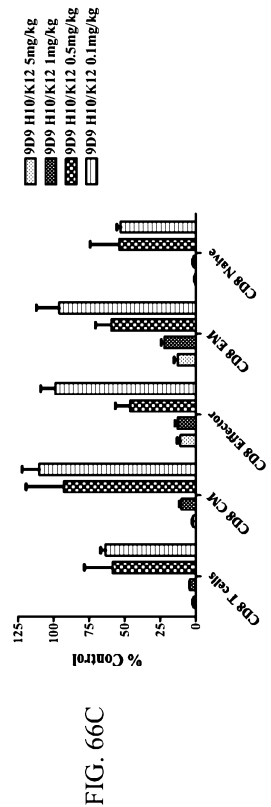
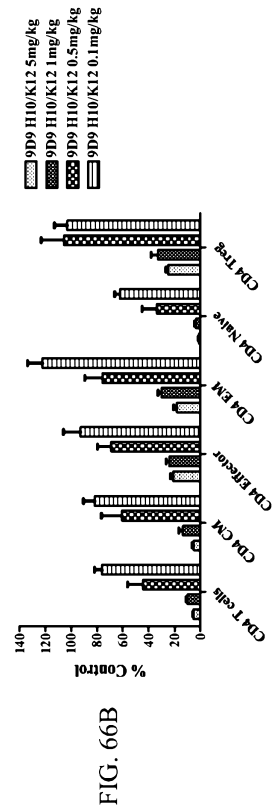


FIG. 65C







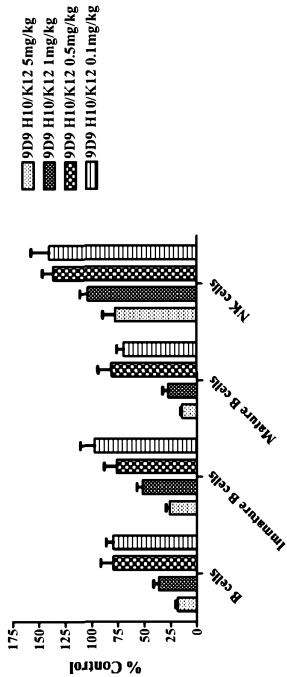


FIG. 66D

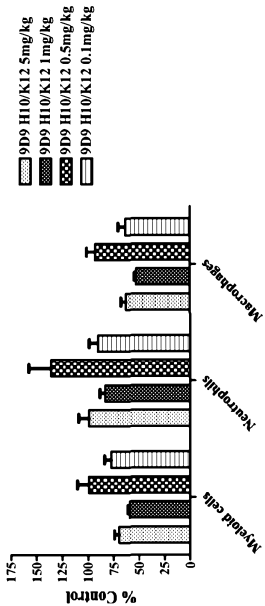


FIG. 66E

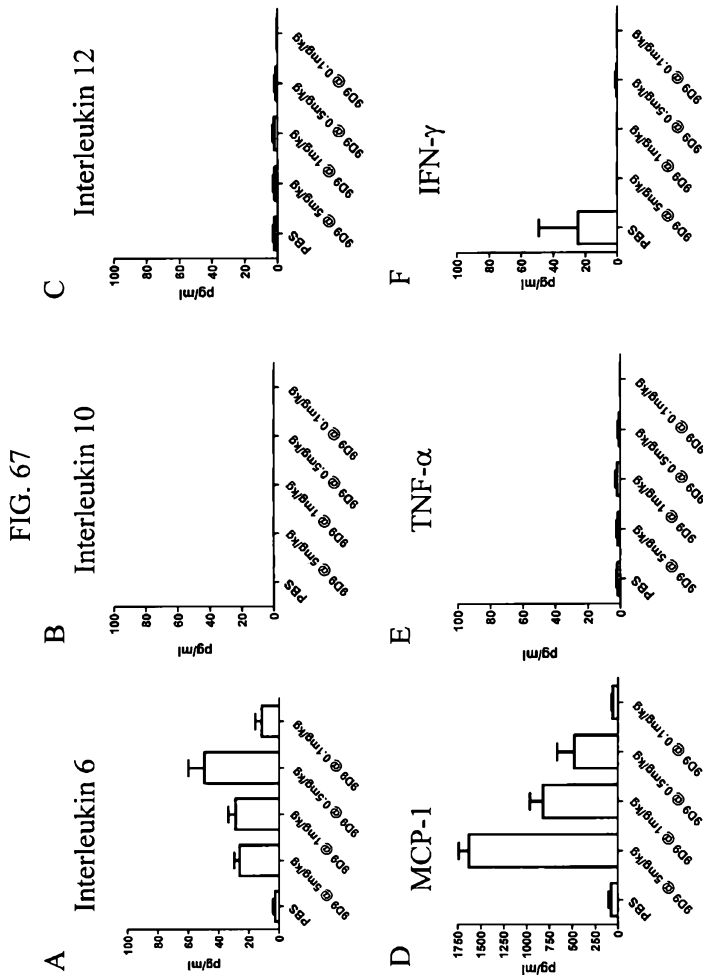
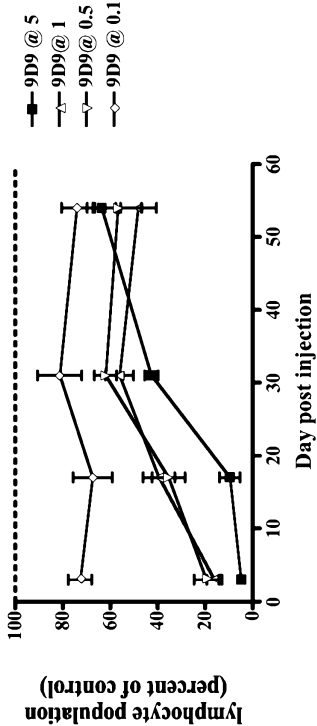
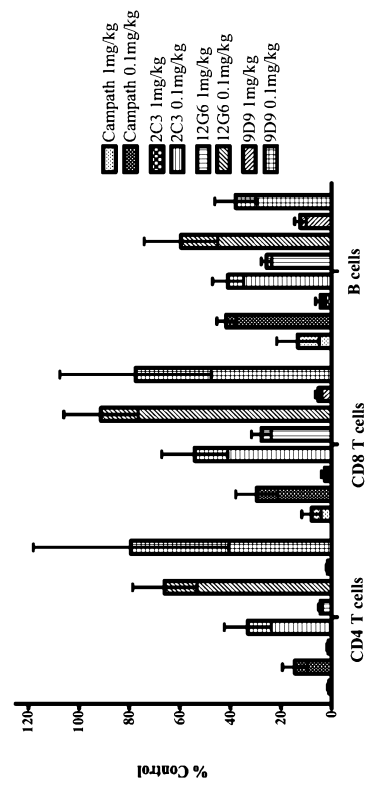


FIG. 68



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FIG. 69A



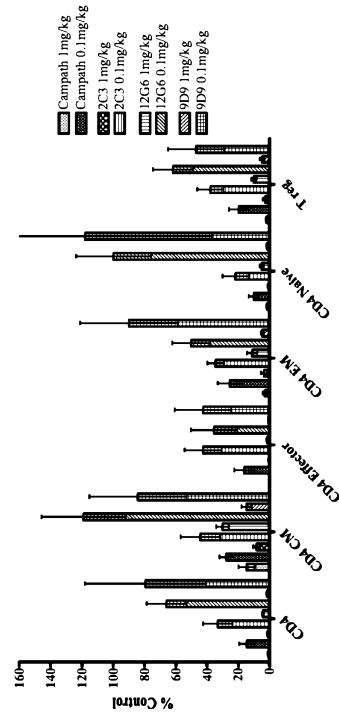


FIG. 69B

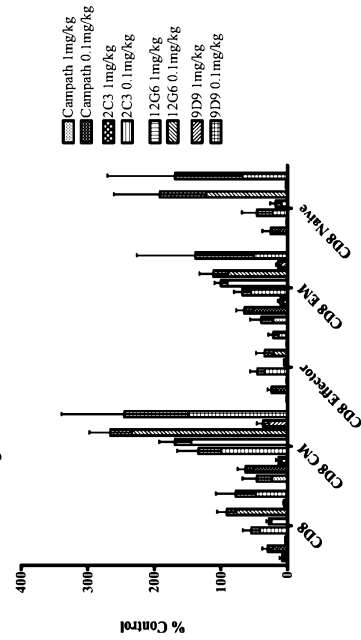
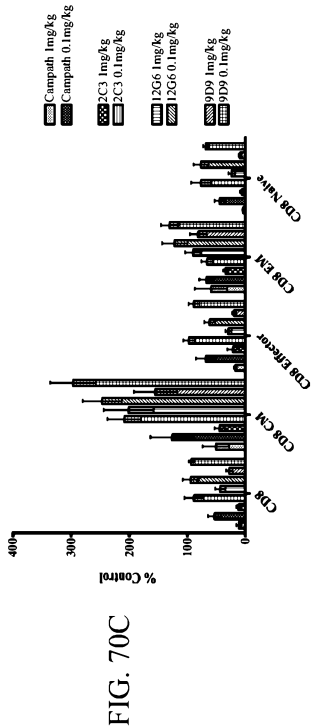
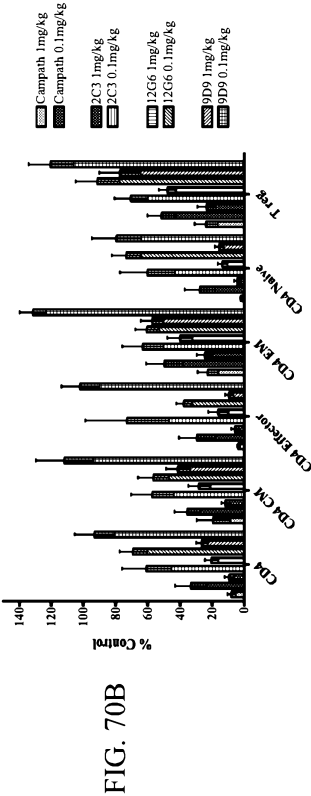
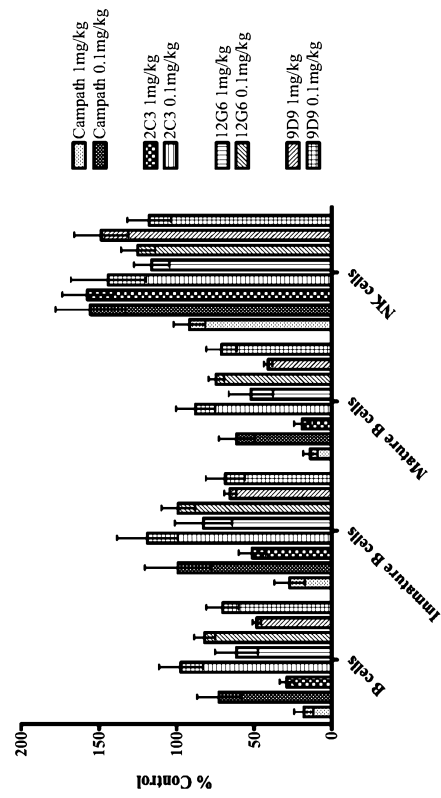


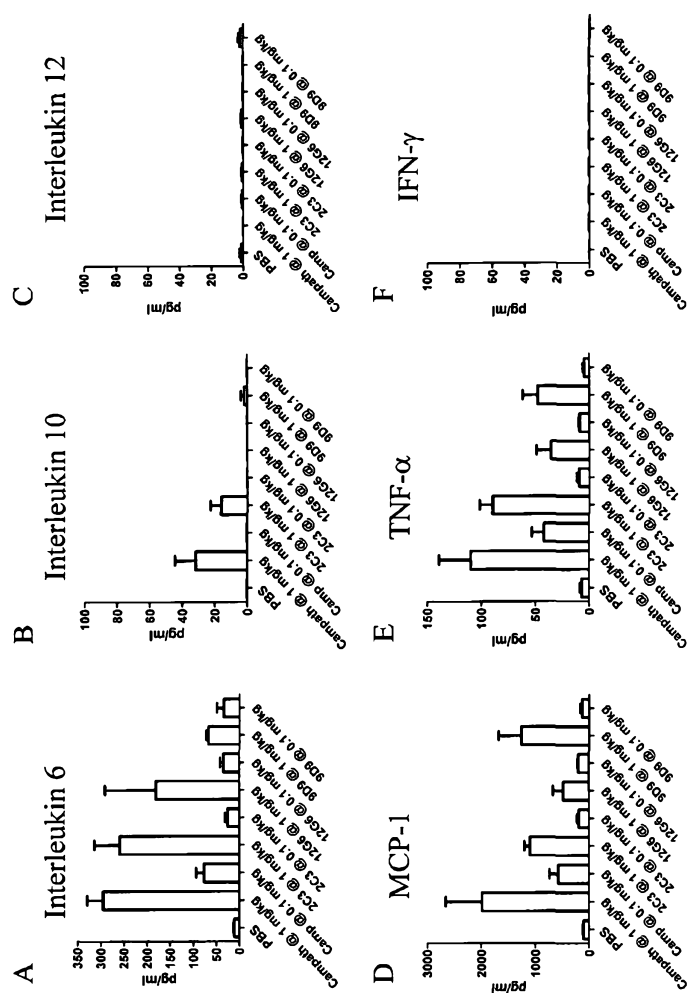
FIG. 69C



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FIG. 70D





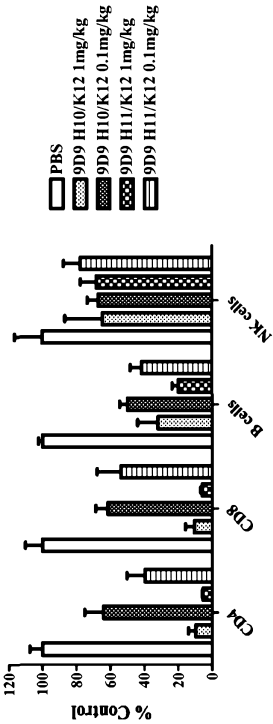


FIG. 72

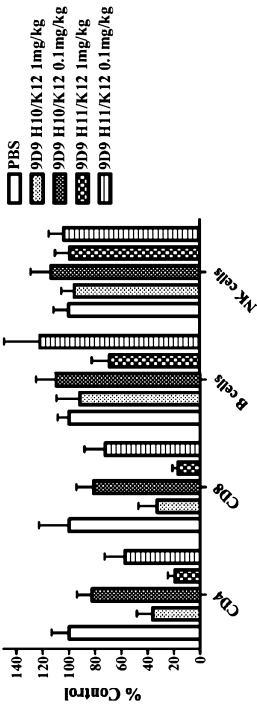


FIG. 73

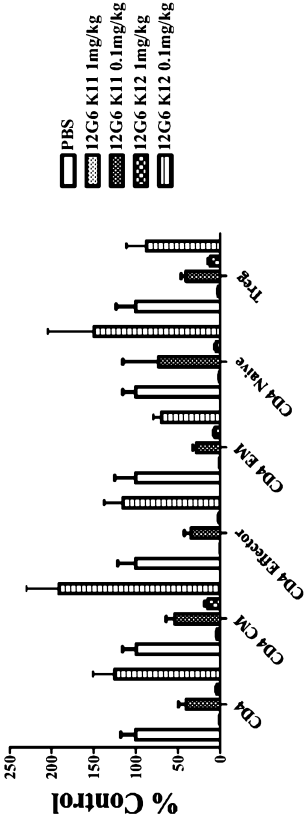


FIG. 74A

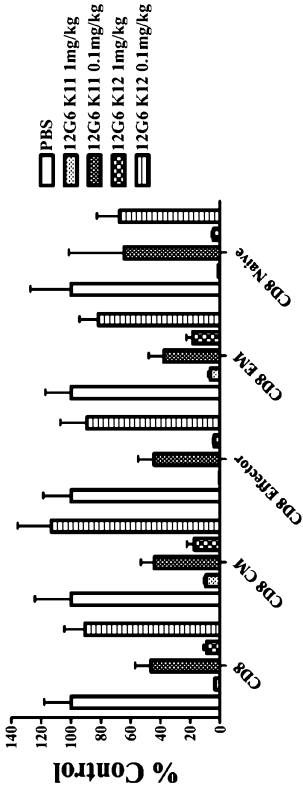
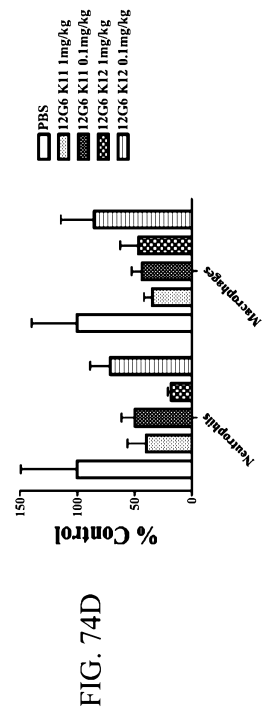
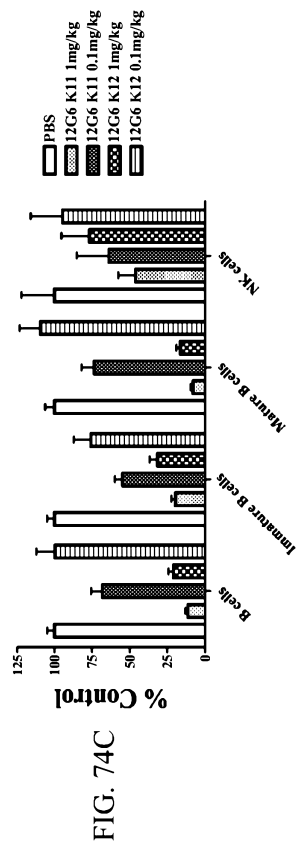
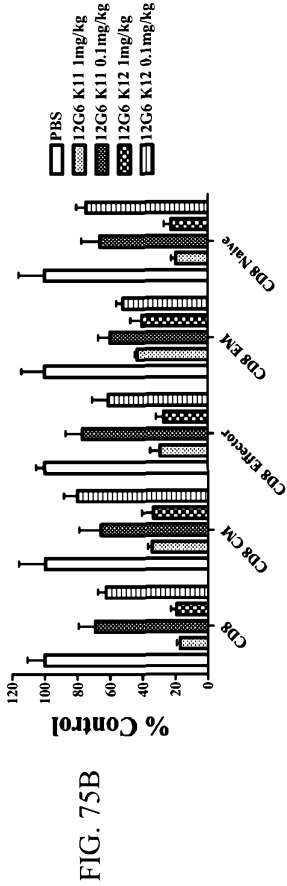
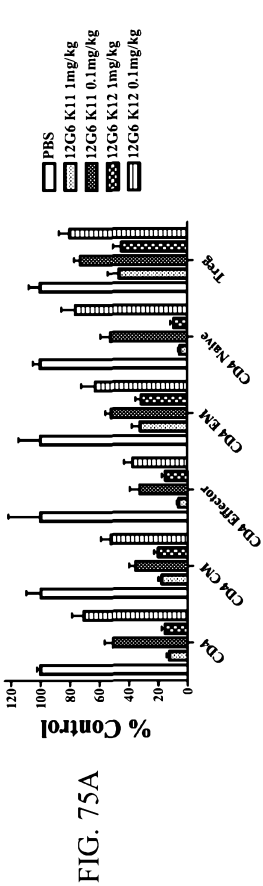


FIG. 74B





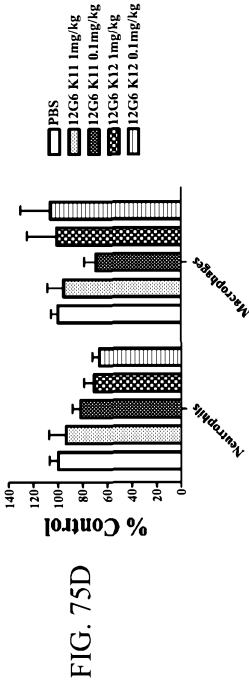
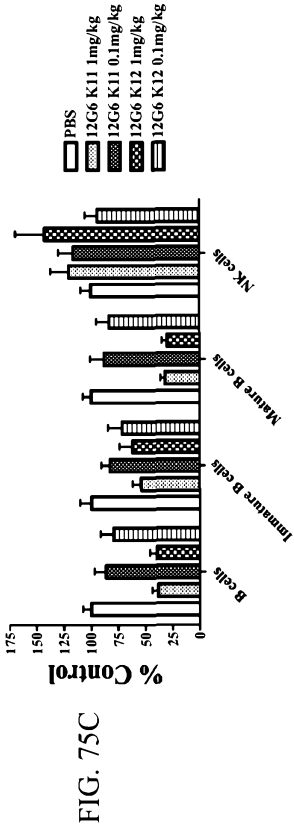
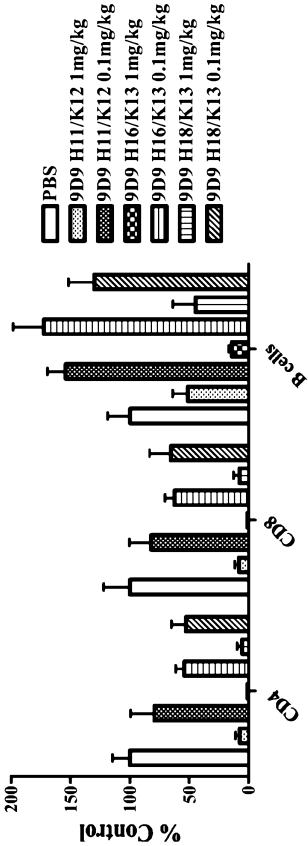


FIG. 76



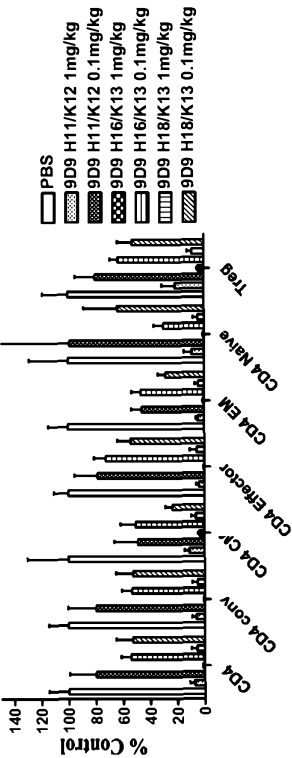


FIG. 77A

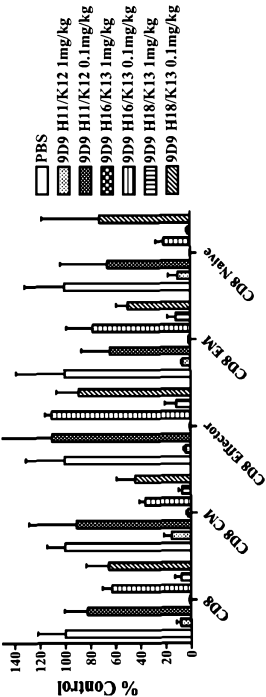


FIG. 77B

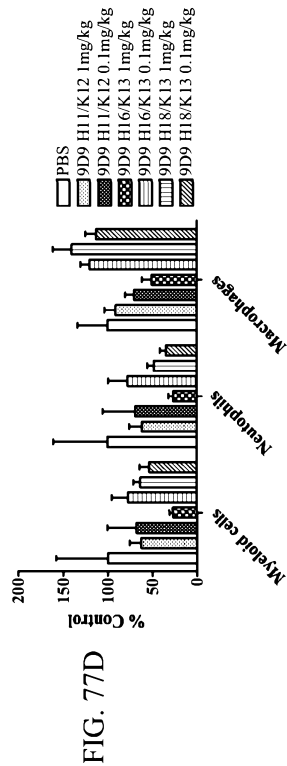
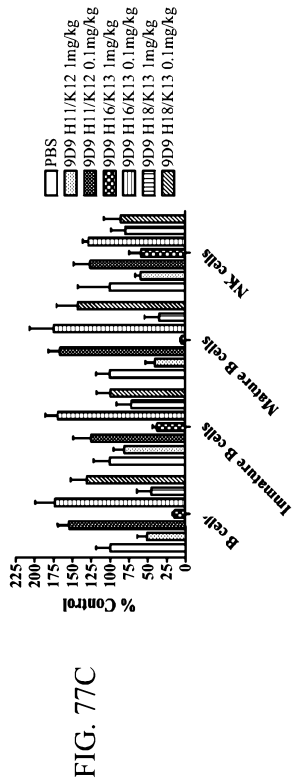
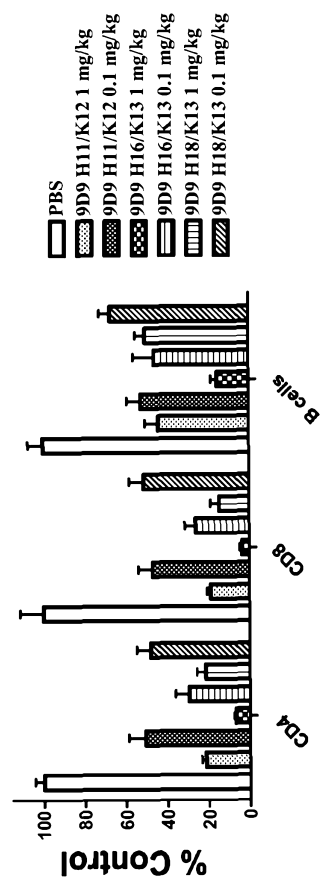


FIG. 78



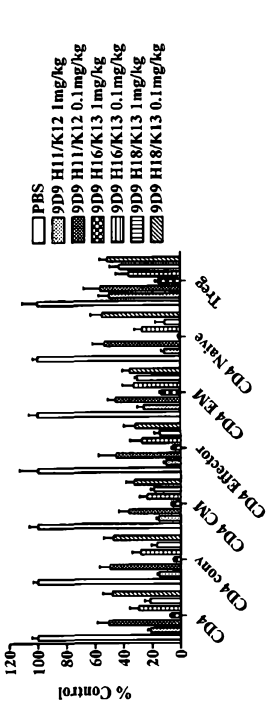


FIG. 79A

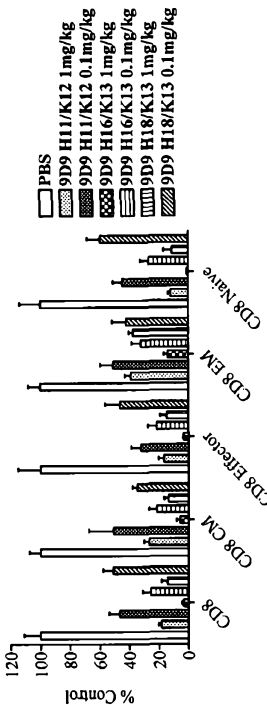


FIG. 79B

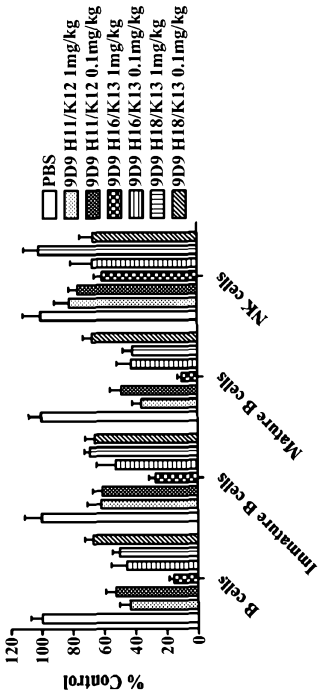


FIG. 79C

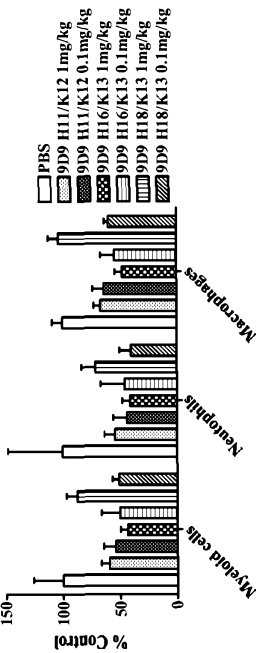
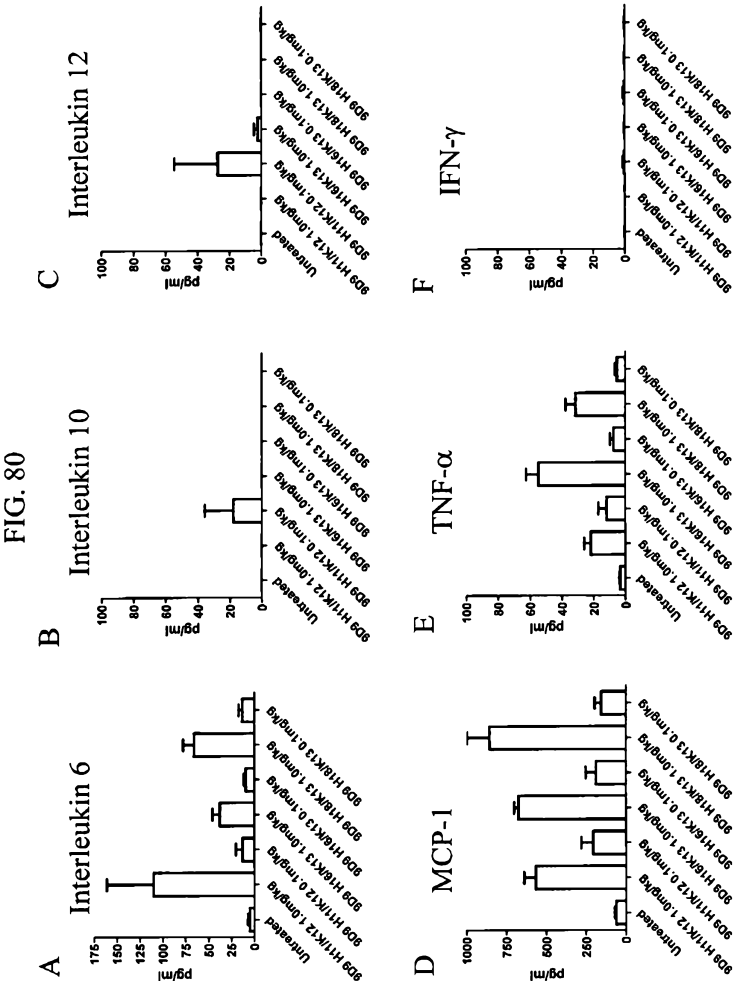
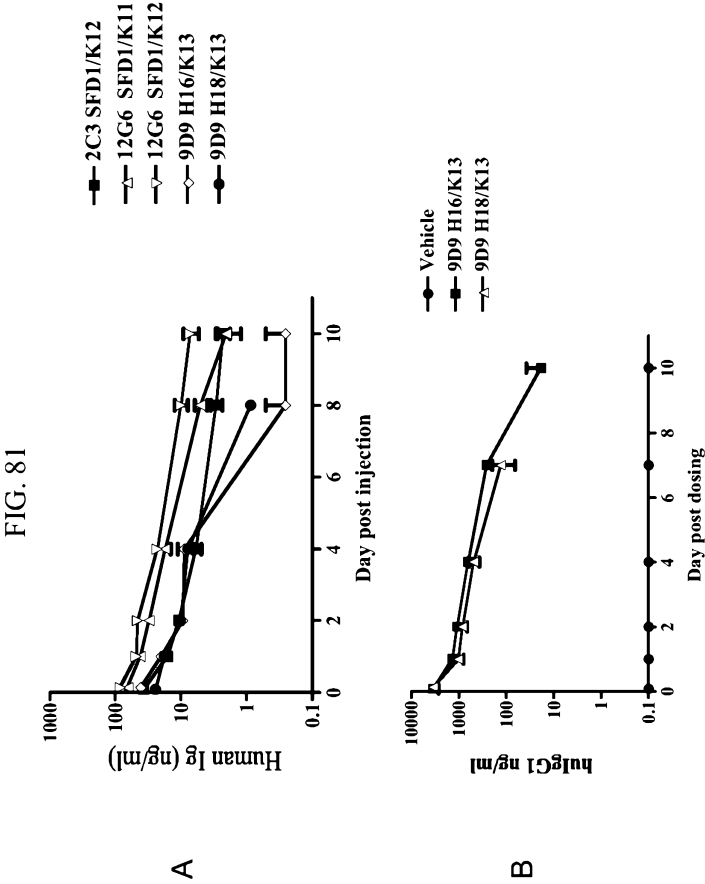
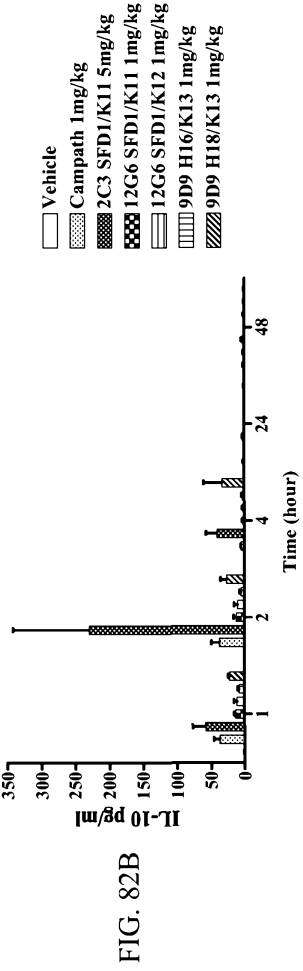
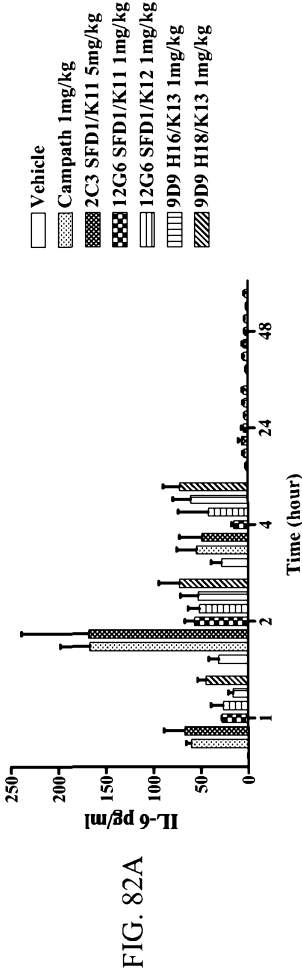
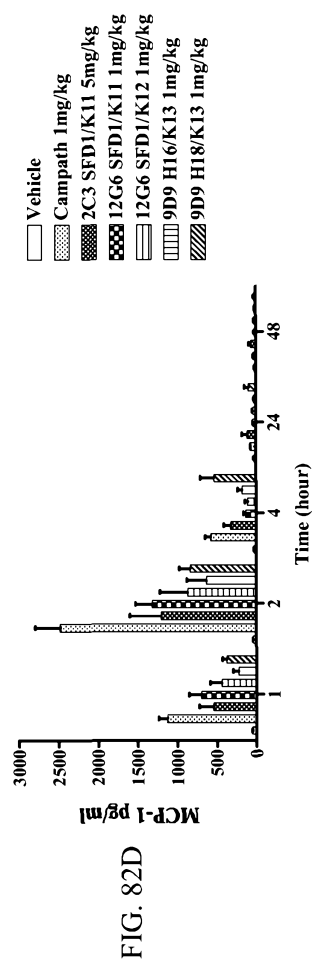
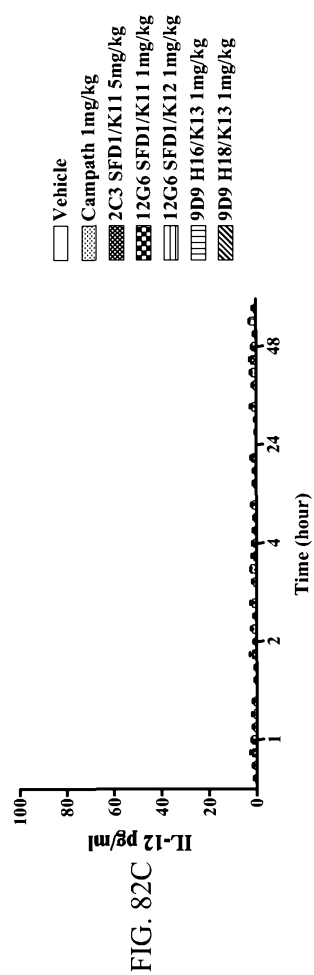


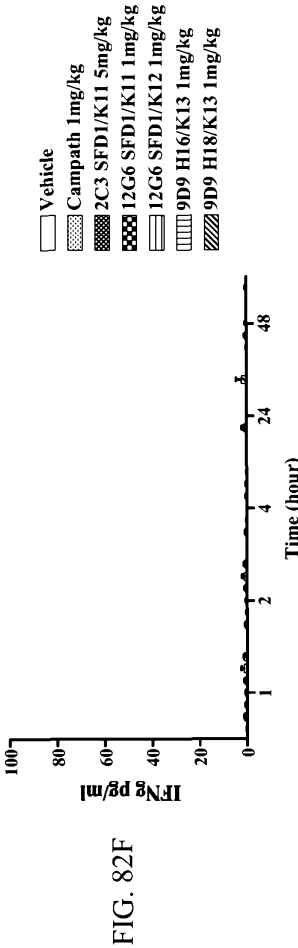
FIG. 79D











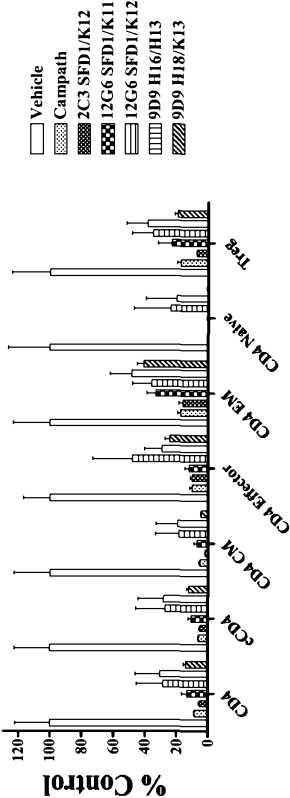


FIG. 83A

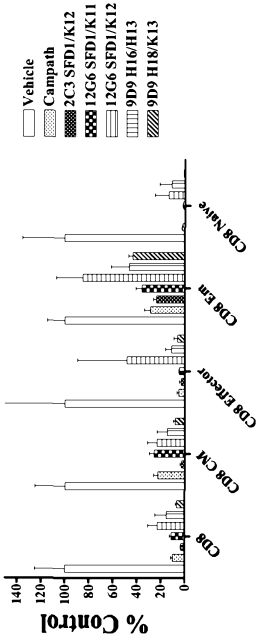


FIG. 83B

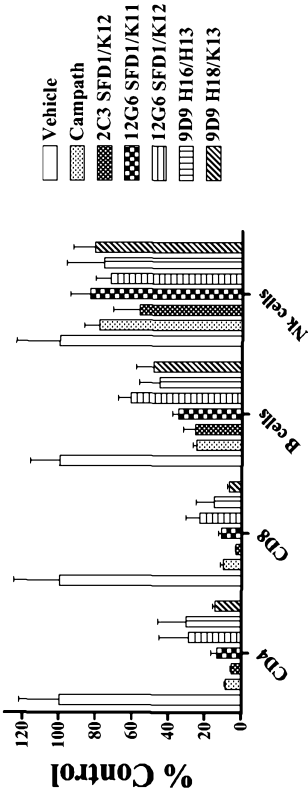


FIG. 83C

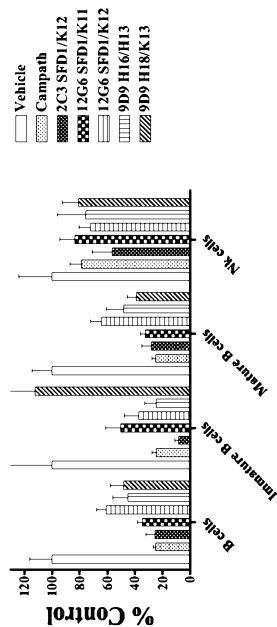


FIG. 83D

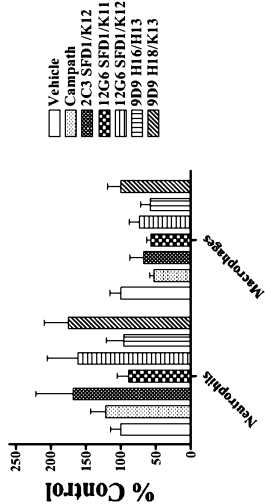


FIG. 83E

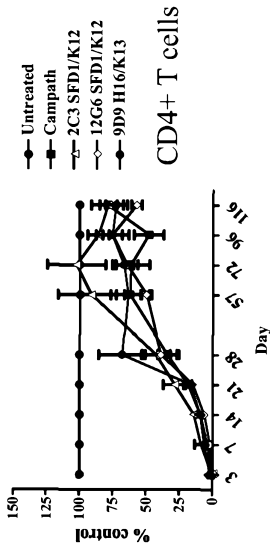


FIG. 84A

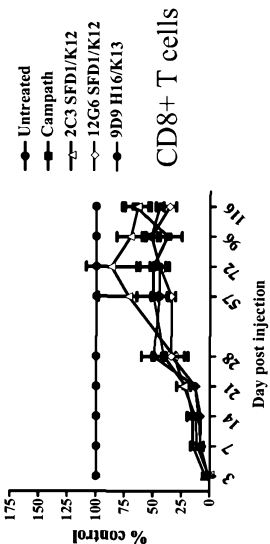


FIG. 84B

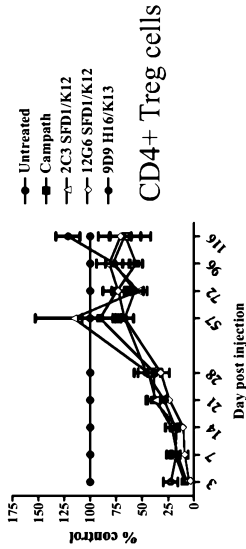


FIG. 84C

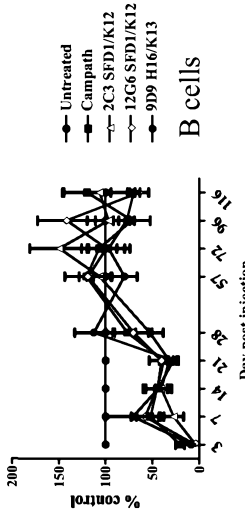


FIG. 84D

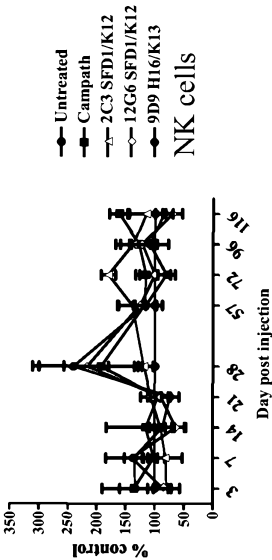


FIG. 84E

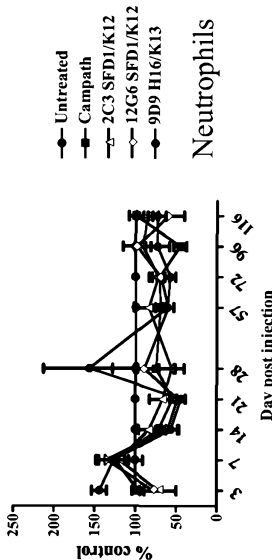
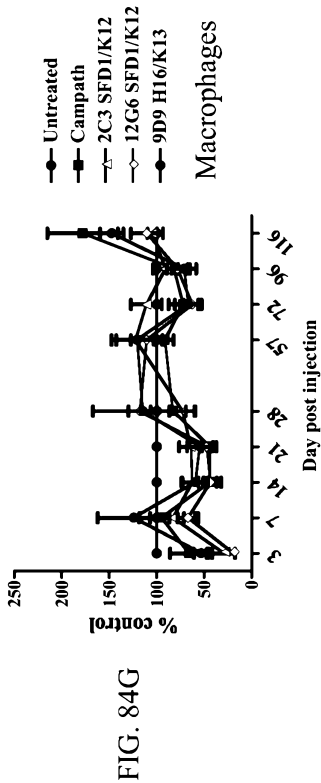


FIG. 84F



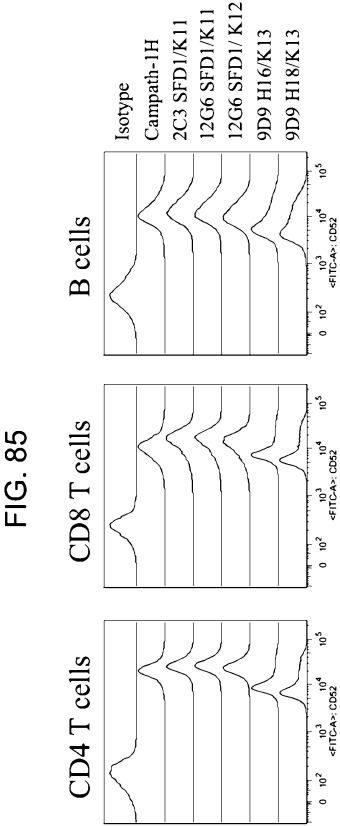
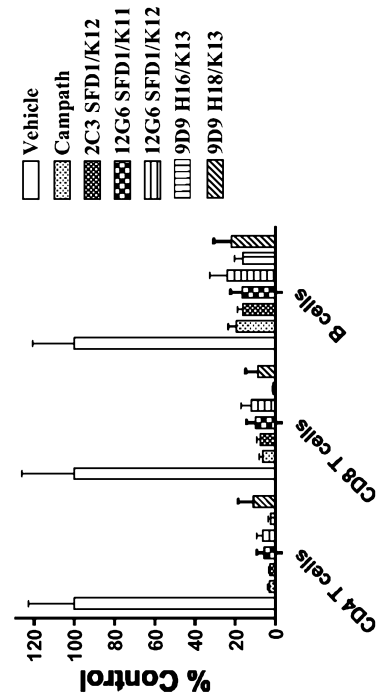


FIG. 86A



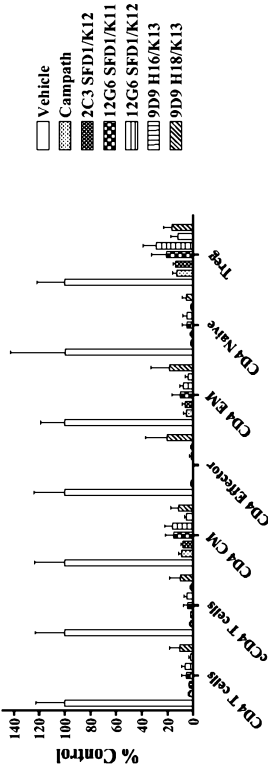


FIG. 86B

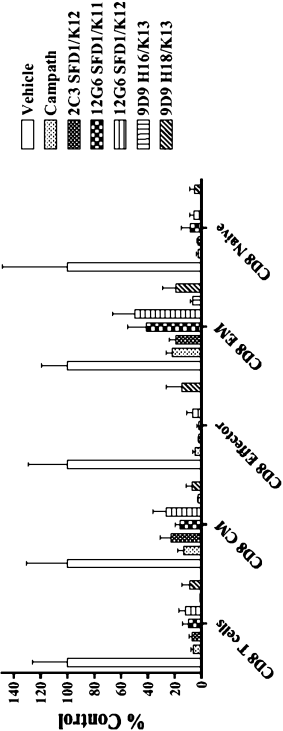


FIG. 86C

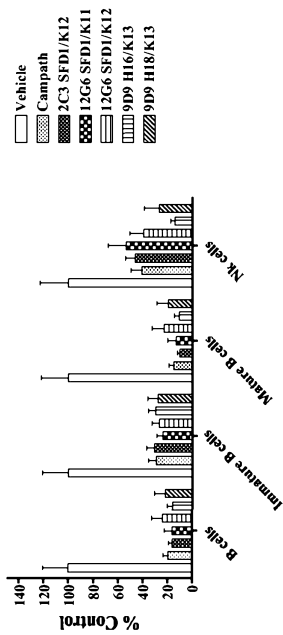


FIG. 86D

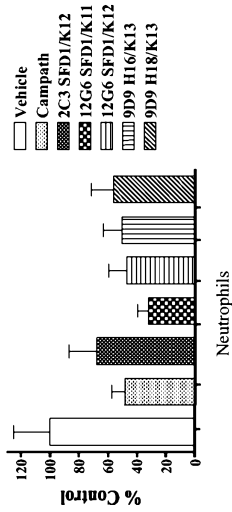
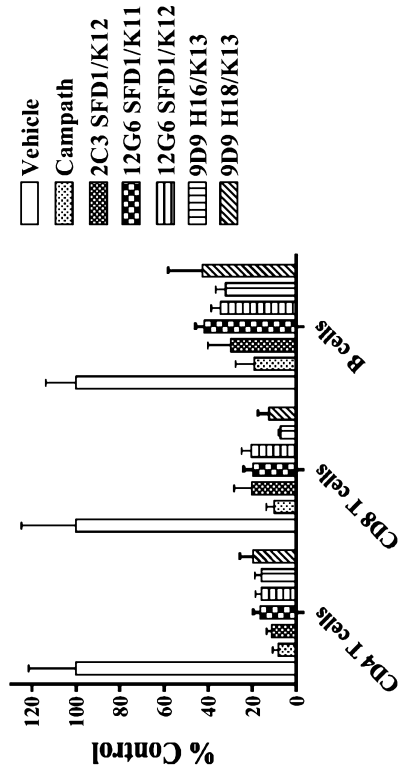


FIG. 86E

FIG. 87A



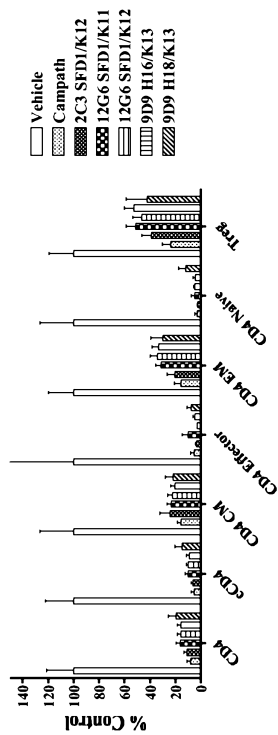


FIG. 87B

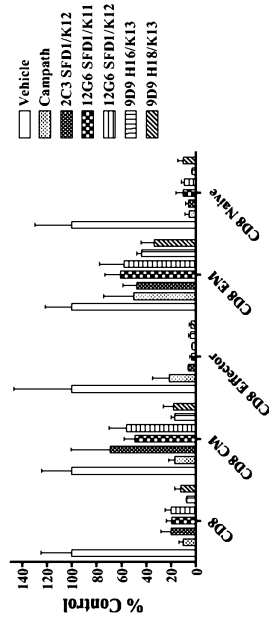


FIG. 87C

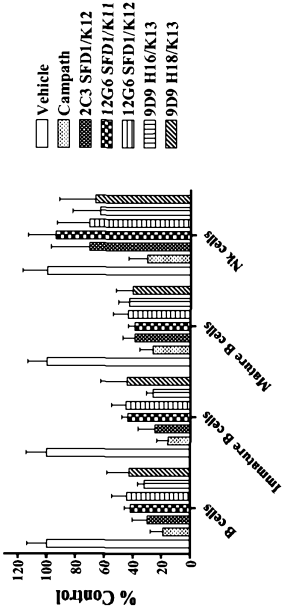


FIG. 87D

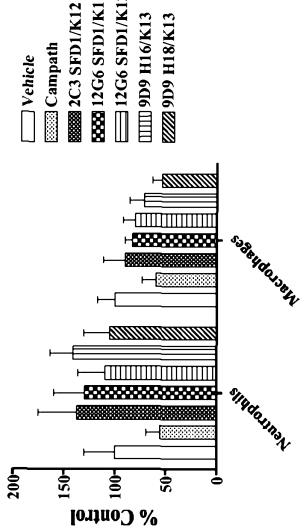
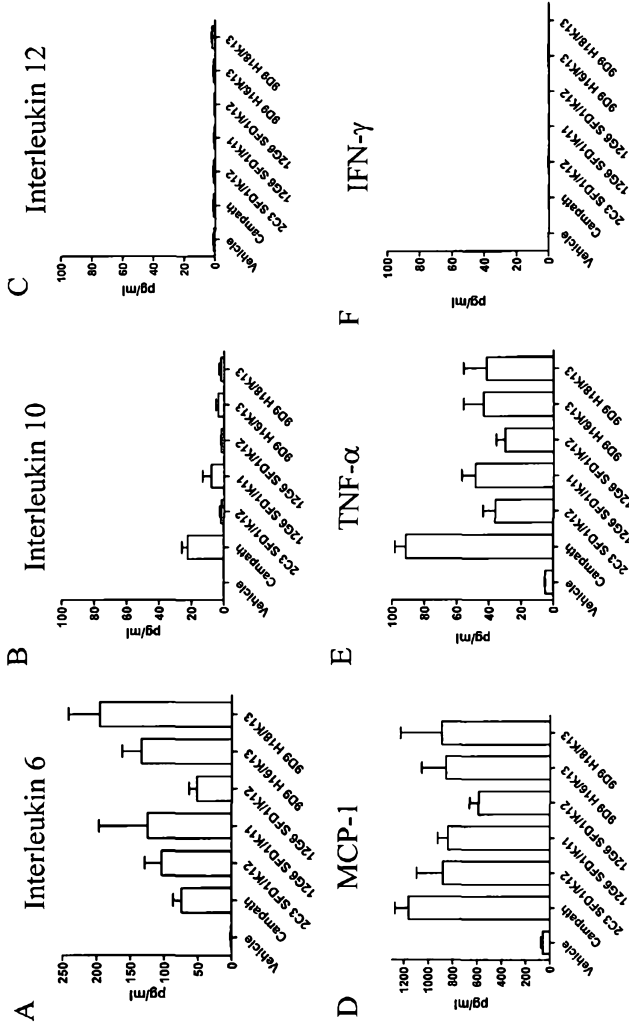


FIG. 87E

FIG. 88



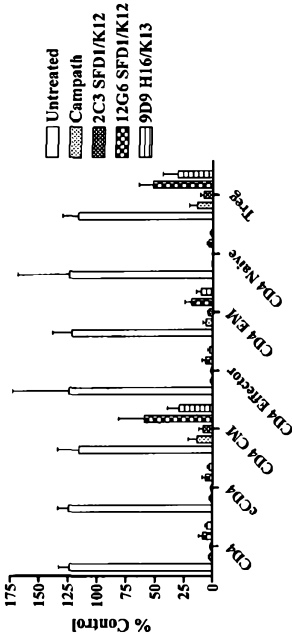


FIG. 89A

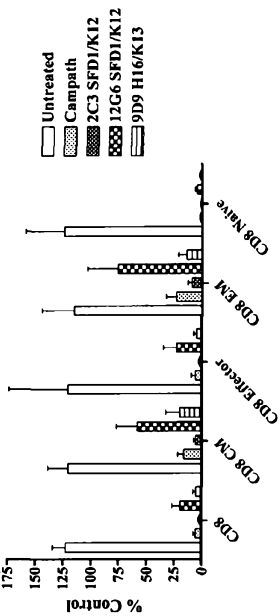


FIG. 89B

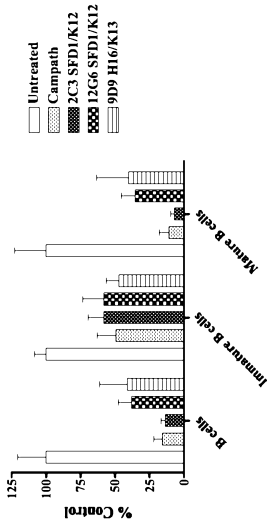


FIG. 89C

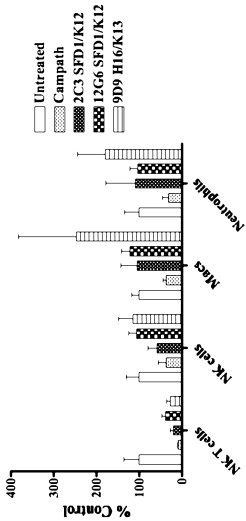


FIG. 89D

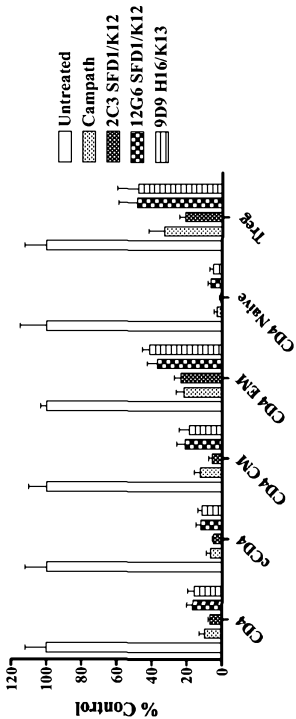


FIG. 90A

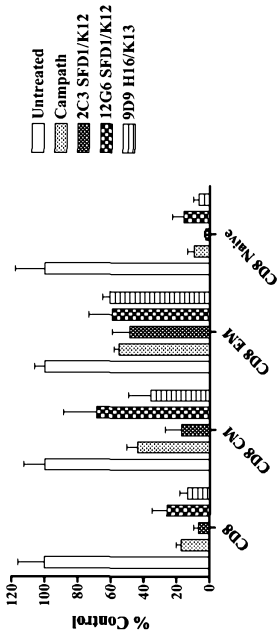


FIG. 90B

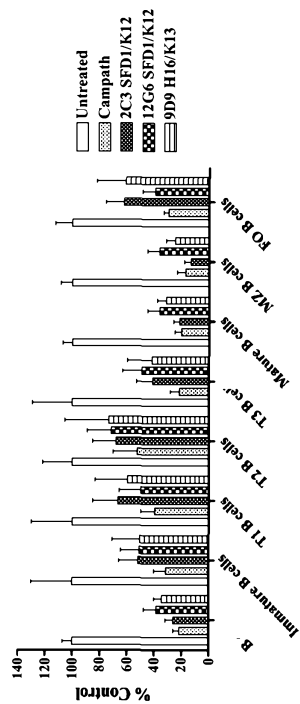


FIG. 90C

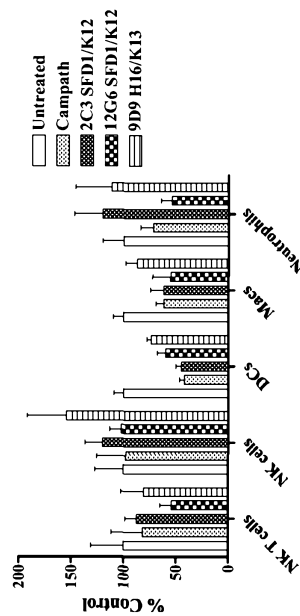


FIG. 90D

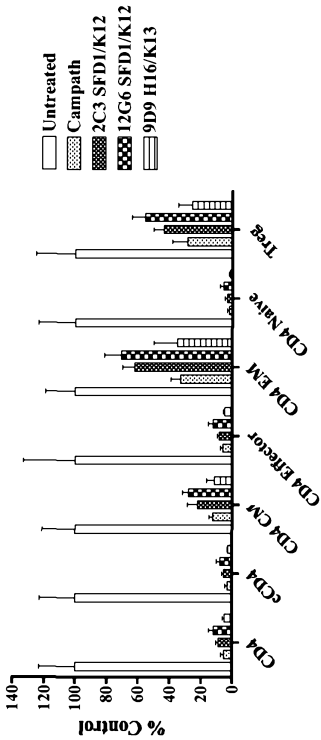


FIG. 91A

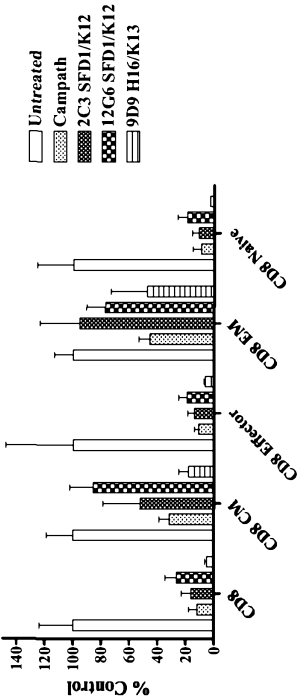


FIG. 91B

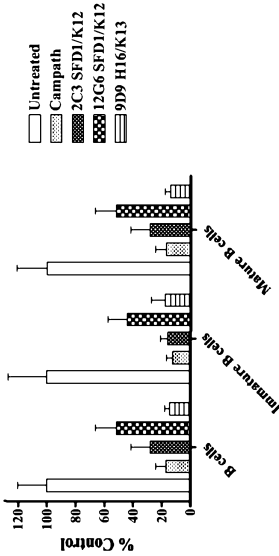


FIG. 91C

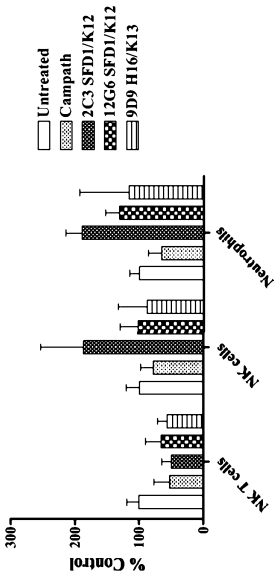
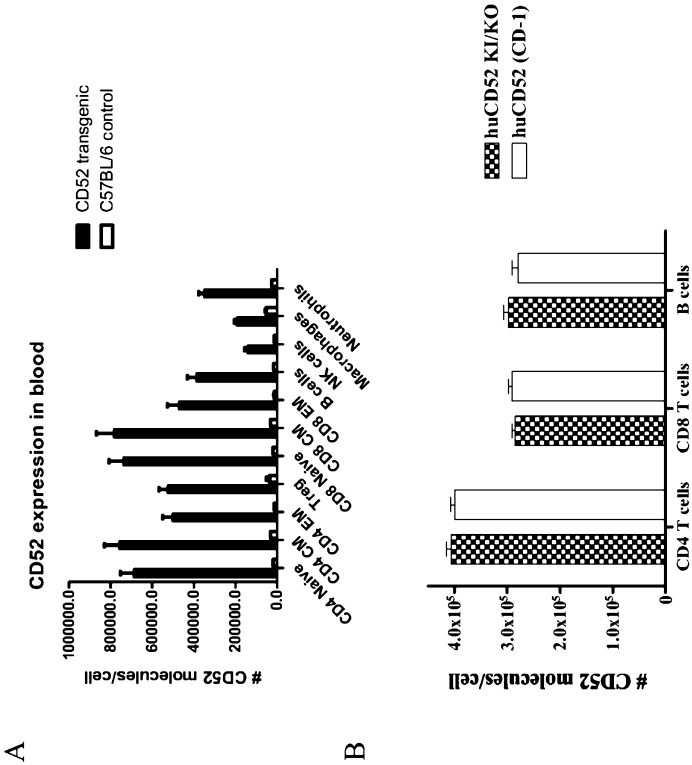


FIG. 91D

FIG. 92



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FIG. 93

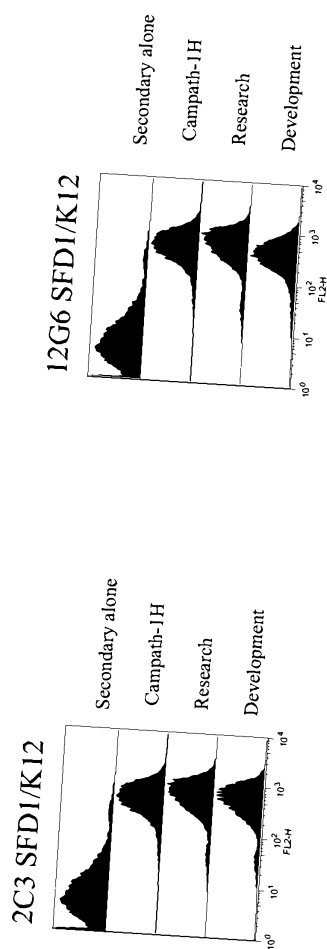


FIG. 94

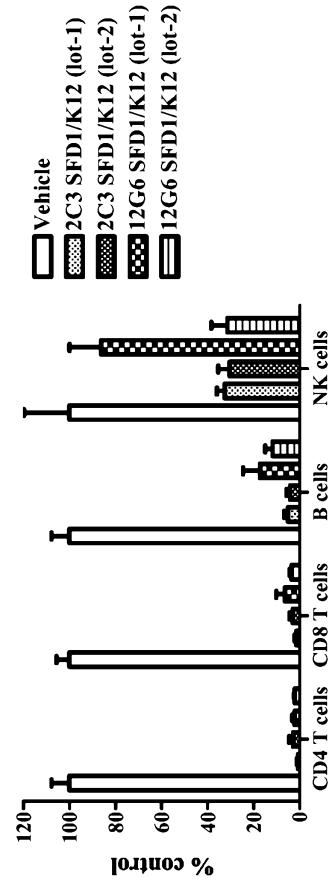
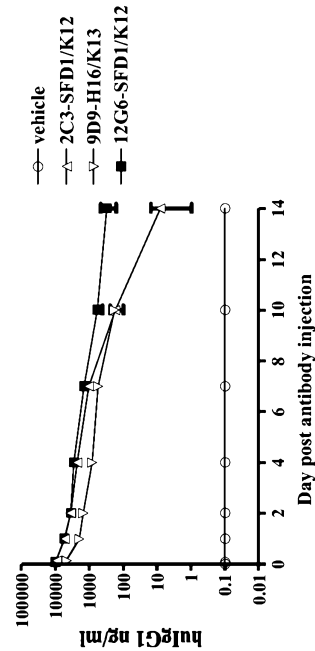


FIG. 95



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FIG. 96

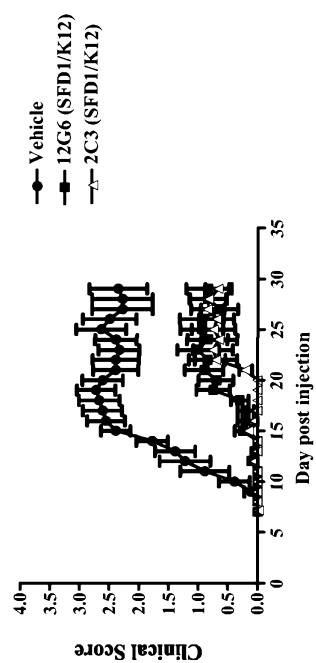


FIG. 97A

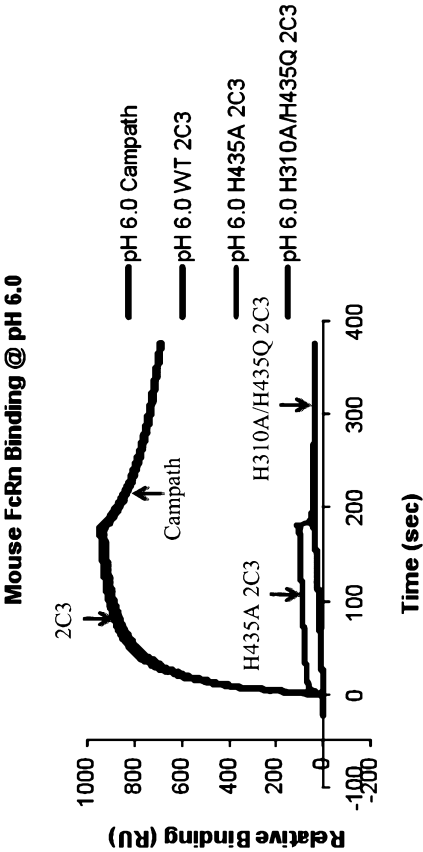
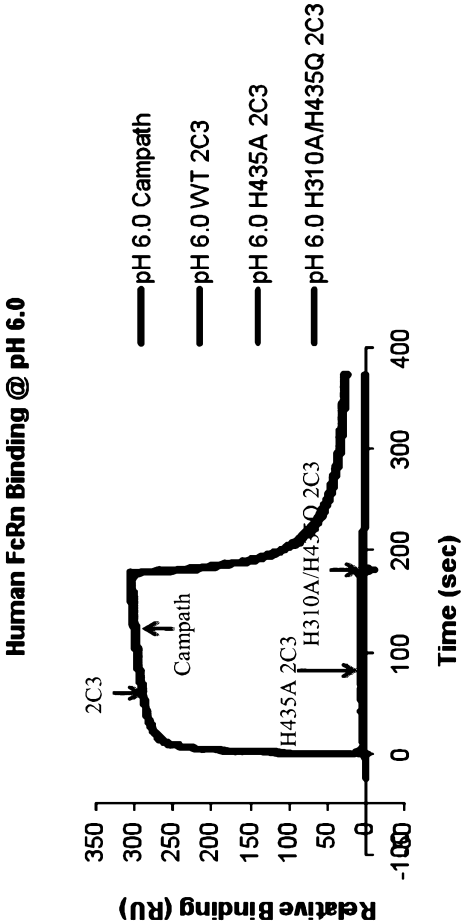
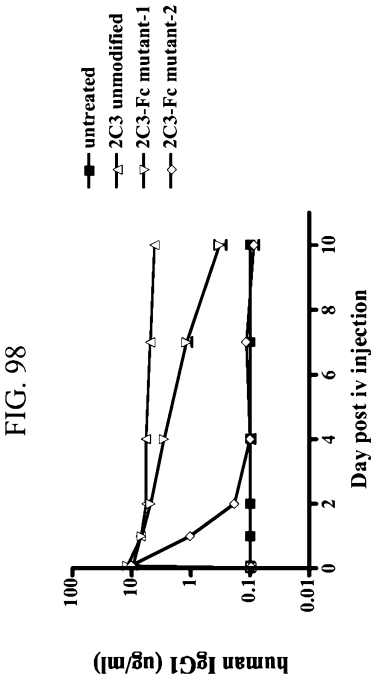


FIG. 97B





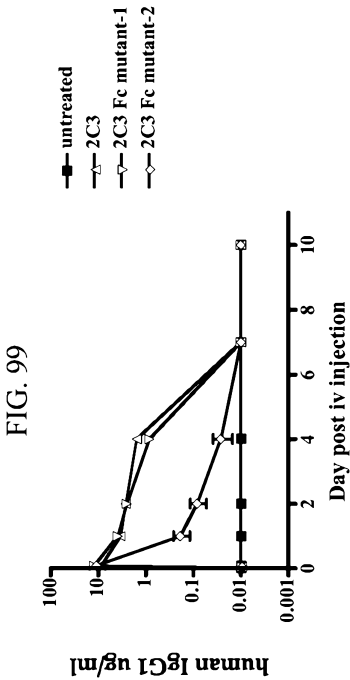
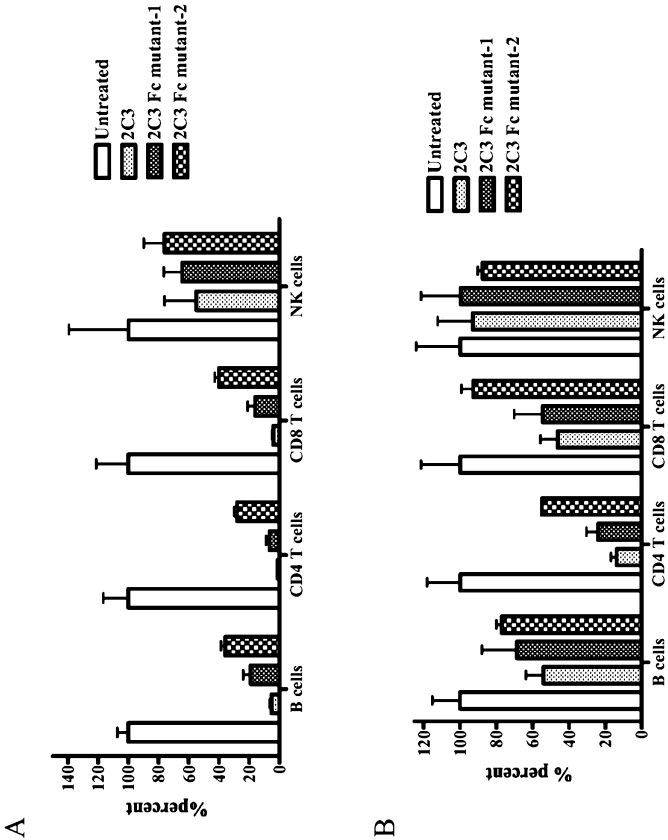


FIG. 100



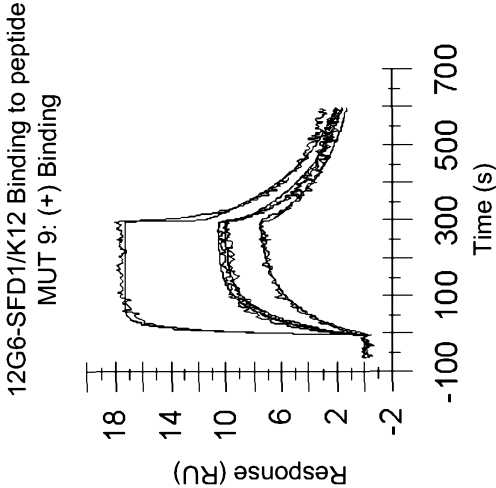


FIG. 101B

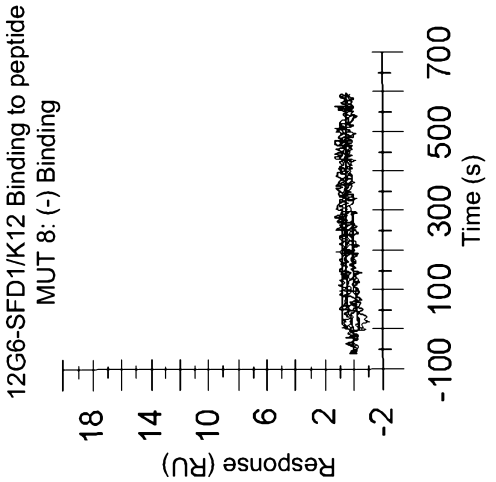


FIG. 101A

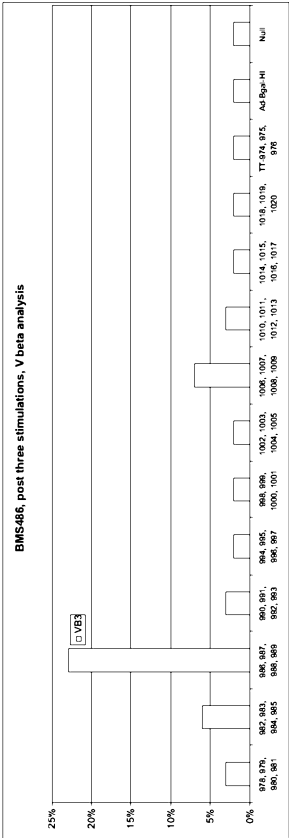


FIG. 102

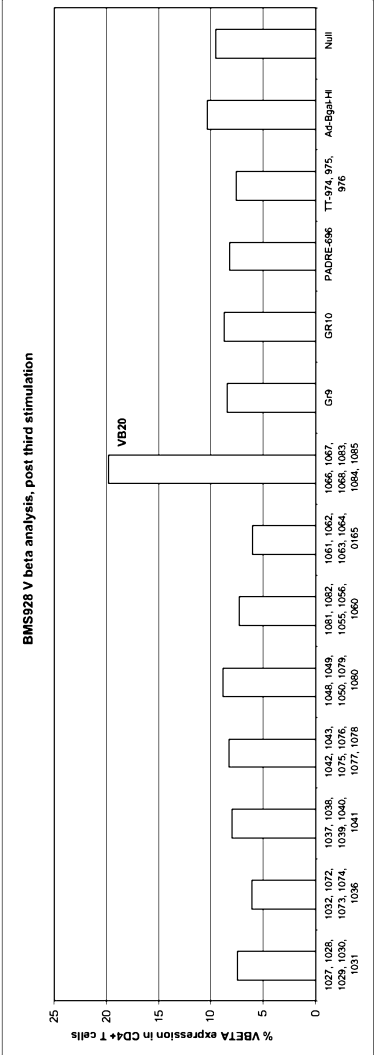


FIG. 103

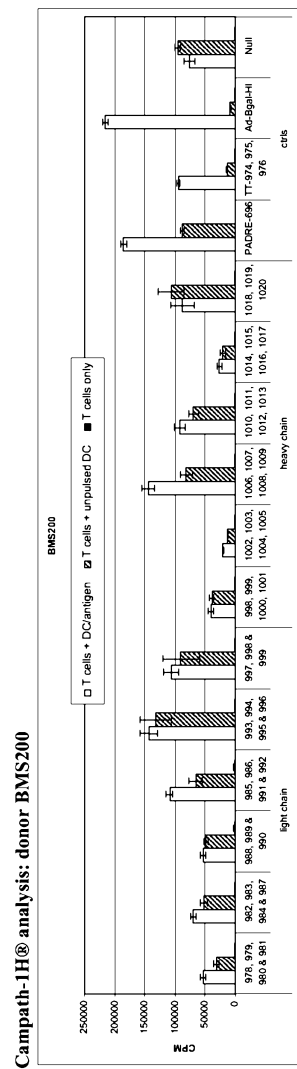


FIG. 104A

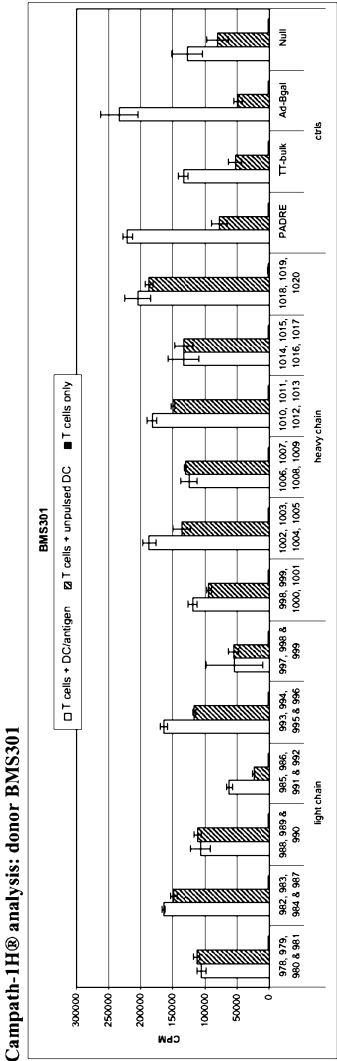


FIG. 104B

Campath-1H® analysis: donor BMS154

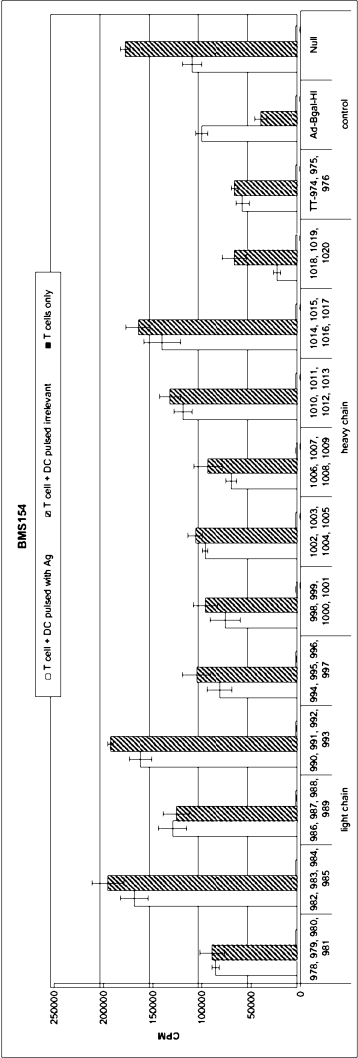


FIG. 104C

Campath-1H® analysis: donor BMS484

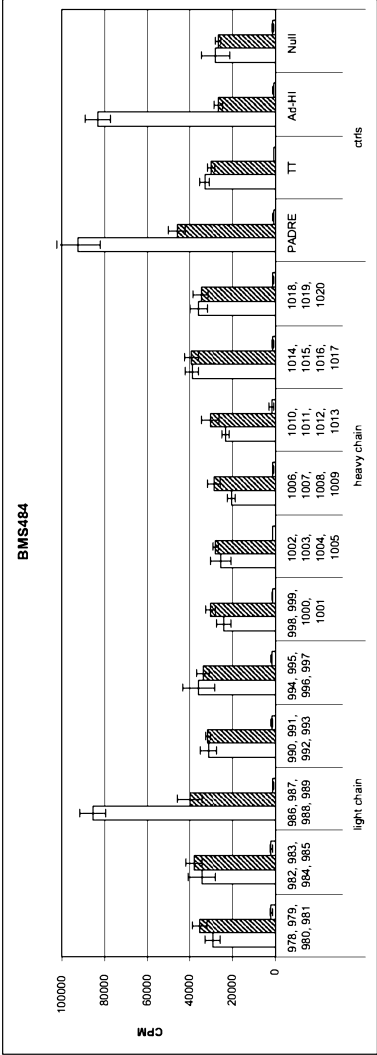


FIG. 104D

Campath-1H® analysis: donor BMS362

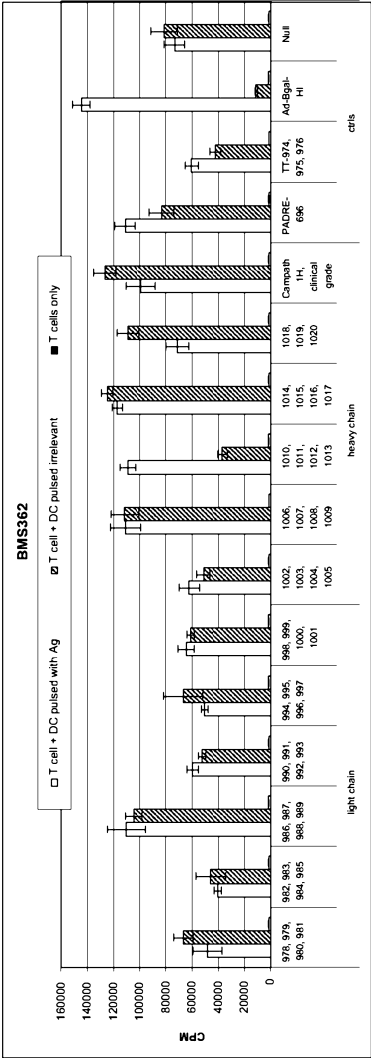


FIG. 104E

Campath-1H® analysis: donor BMS486

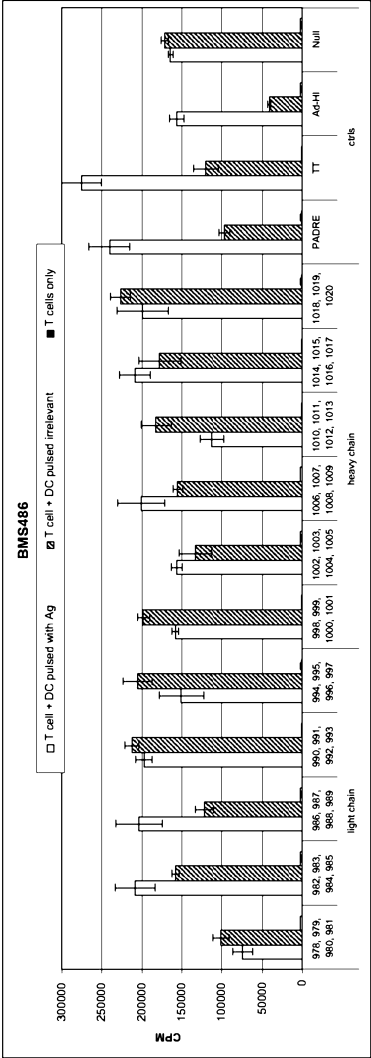


FIG. 104F

Campath-1H® analysis: donor BMS150

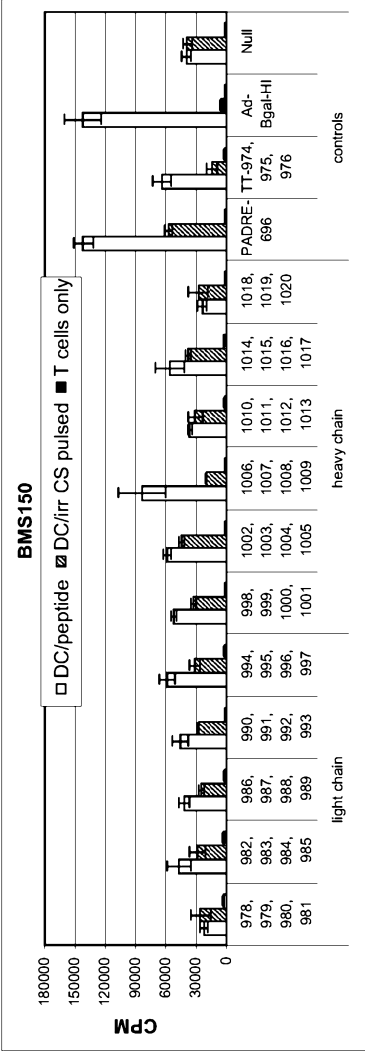


FIG. 104G

Campath-1H® analysis: donor BMS167

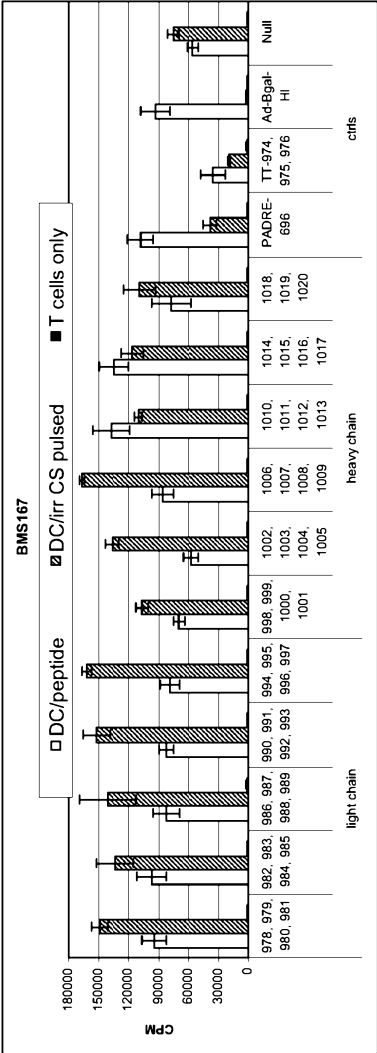


FIG. 104H

Campath-1H® analysis: donor BMS170

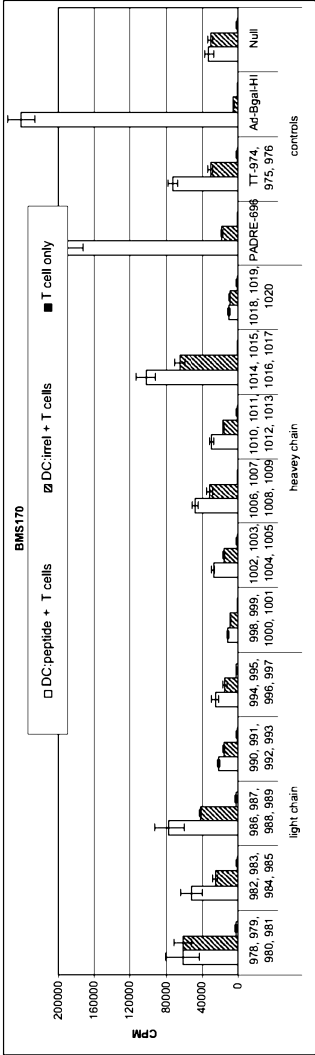


FIG. 104I

Campath-1H® analysis: donor BMS352

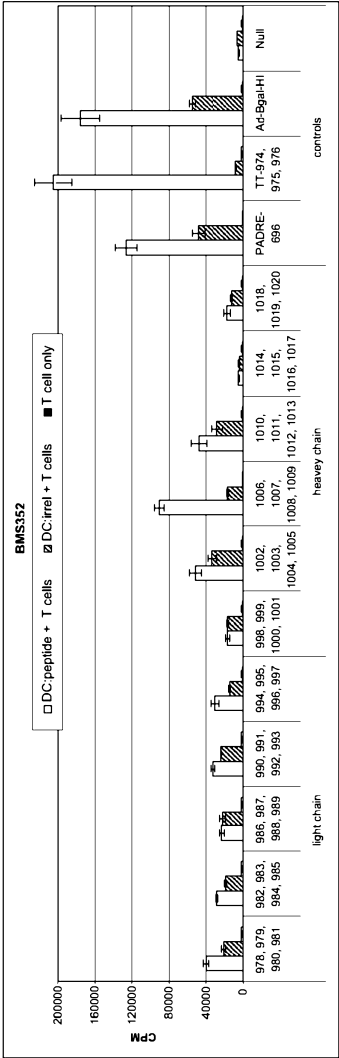


FIG. 104J

12C6-SFD1/K12 analysis: donor BMS484

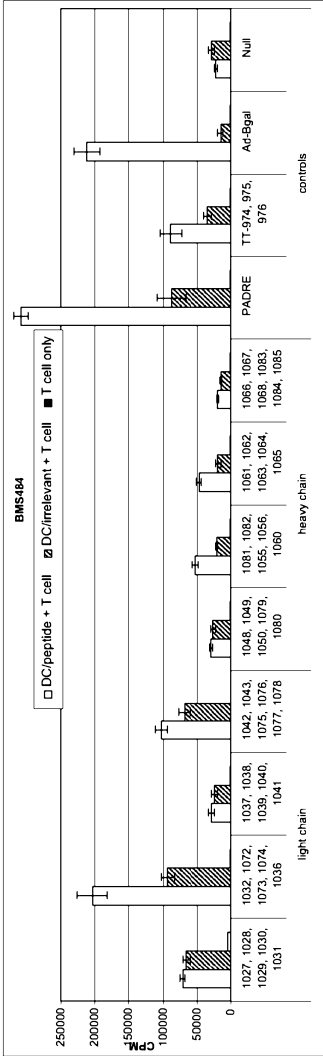
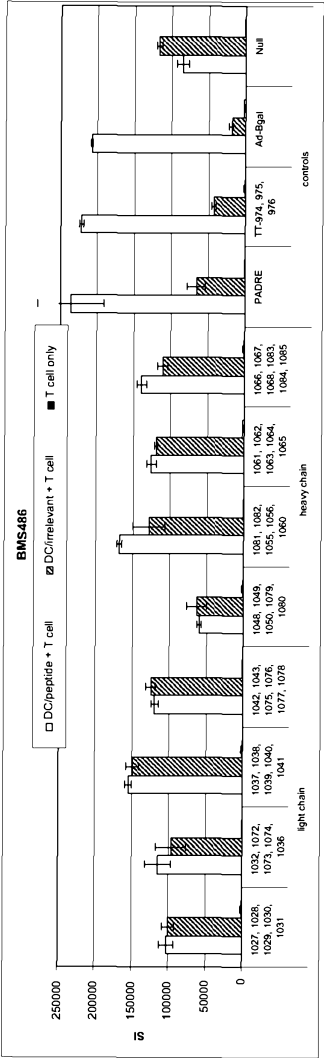


FIG. 105A

12G6-SFDI/K12 analysis: donor BMS486



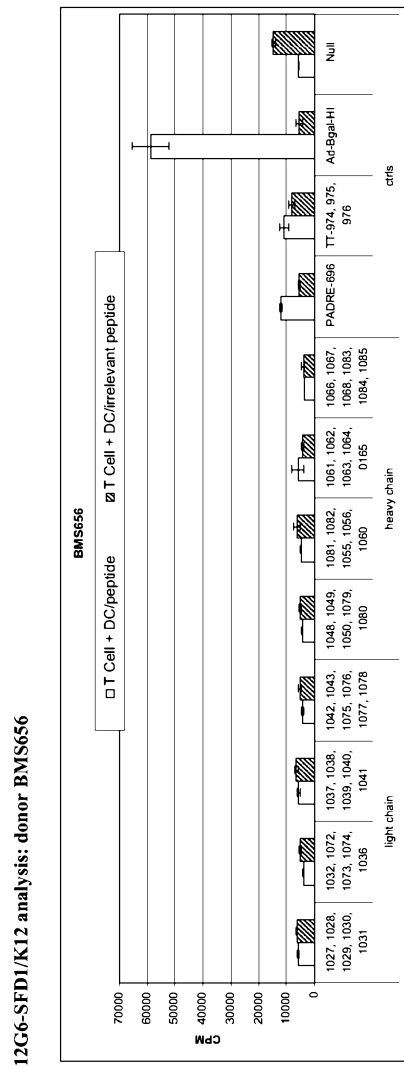


FIG. 105C

12G6-SFD1/K12 analysis: donor BMS640

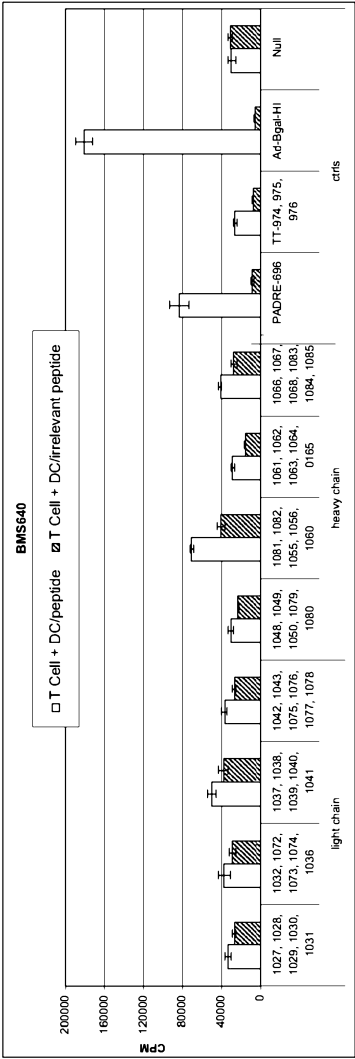


FIG. 105D

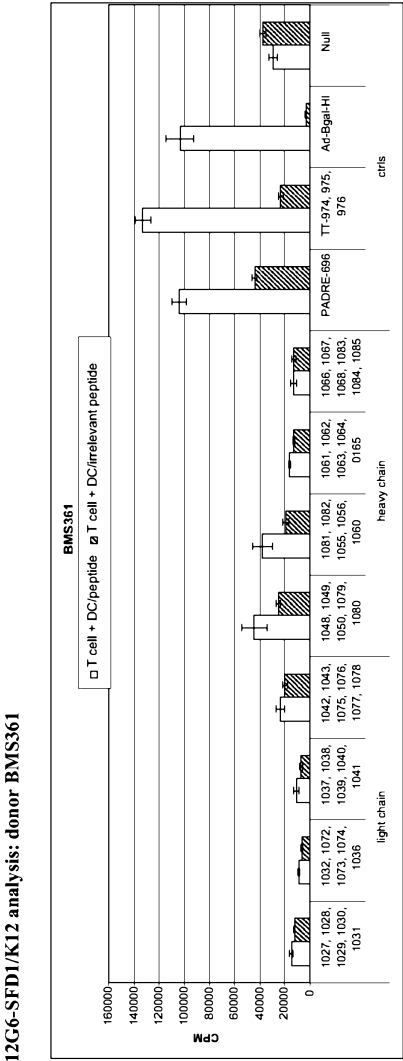


FIG. 105E

12G6-SFD1/K12 analysis: donor BMS165

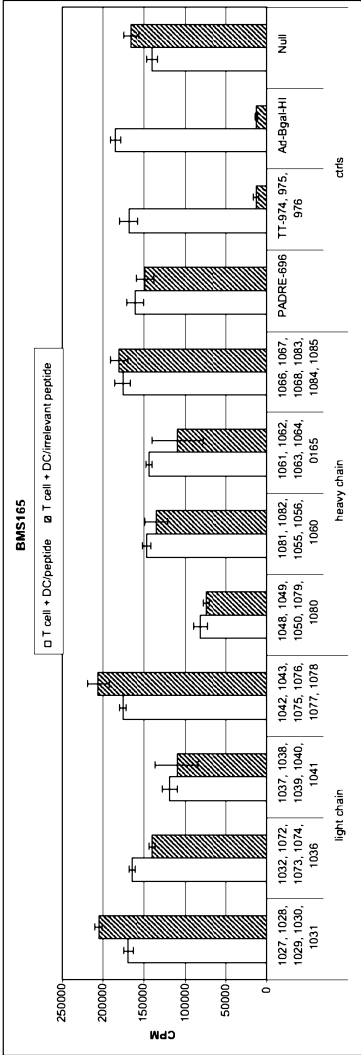


FIG. 105F

12G6-SFD1/K12 analysis: donor BMS902

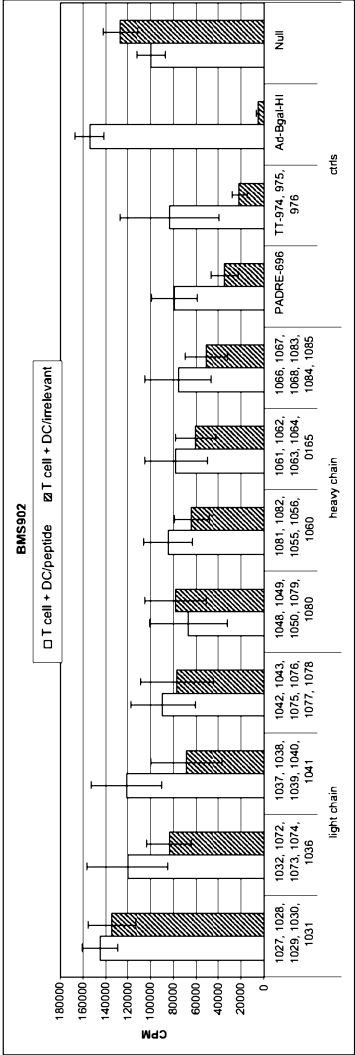


FIG. 105G

12G6-SFD1/K12 analysis: donor BMS928

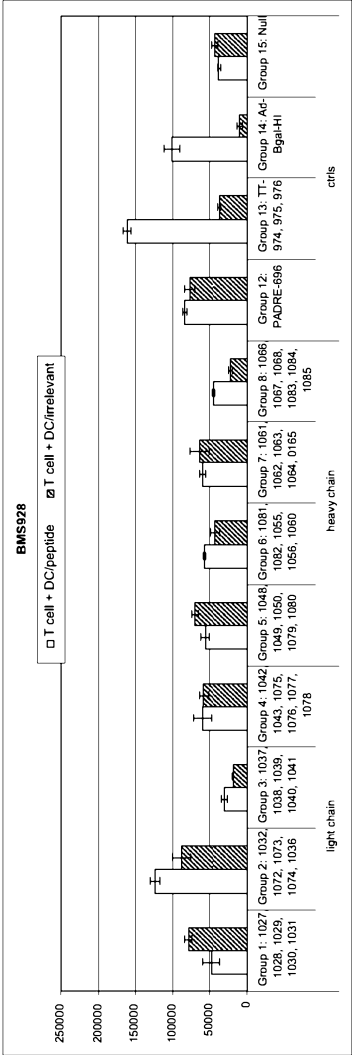


FIG. 105H

12G6-SFD1/K12 analysis: donor BMS927

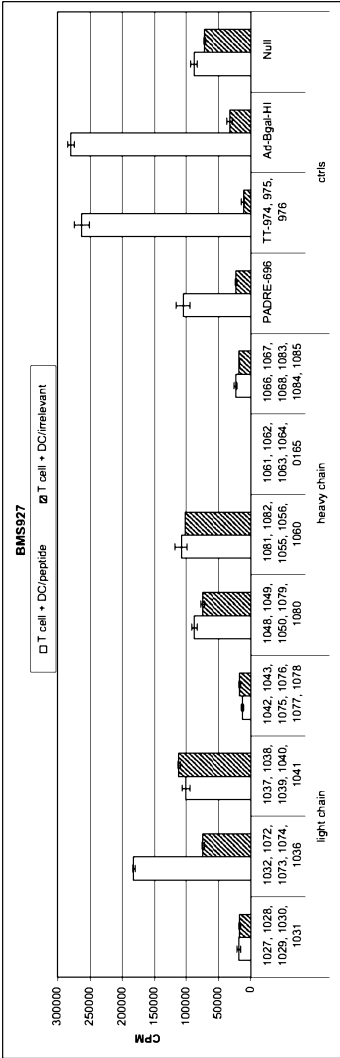


FIG. 105I

12G6-SFD1/K12 analysis: donor BMS963

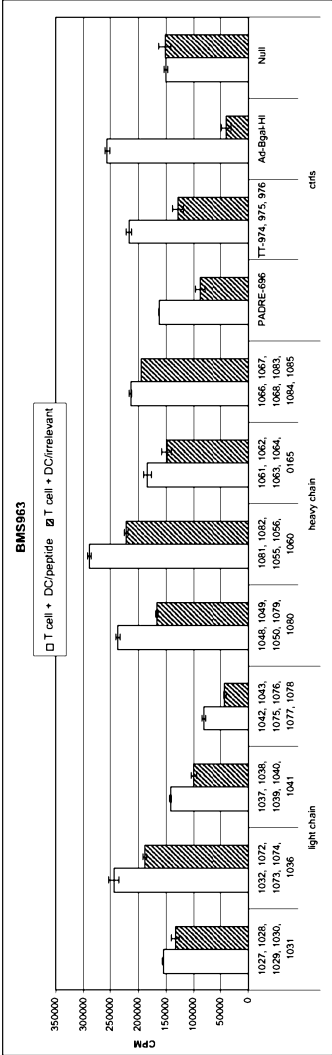


FIG. 105J

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Humanized 2C3 clone full-length heavy chain sequence**2C3-SFD1**

MEAPAQLLFLLLWLPDTTGEVQLVESGGGLVQPGGSLRLSCAASGFTFTYWMNWV
RQAPGKGLEWVGQIRLKSNNYATHYAESVKGRFTISRDDSKNSLYLQMNSLKTEDTAV
YYCTPVDFWGQGTITVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE
PKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPETCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO: 272)

Humanized 2C3 clone full-length light chain sequence**2C3-K12**

MEAPAQLLFLLLWLPDTTGDIVMTQTPLSLSVTPGQPASISCKSSQSLLYSNGKTYL
LLQKPGQSPQRLIYLVSKLDSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGT
HTFGQGRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGE
C (SEQ ID NO: 273)

FIG. 106

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Humanized 7F11 clone full-length heavy chain sequence

7F11-SFD1

MEAPAQLLFLLLLWLPDTTGEVQLVESGGGLVQPGGSLRLSCAASGFTFTDYYMSWV
RQAPGKGLEWVG**FIRNKANGYTTEYNASVKGRFTISRDDSKNSLYLQMNSLKTEDTA**
VYYCTRYIFFDYWGQGT**TTTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP**
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV**VTVPSSSLGTQTYICNVNHKPSNTKV**
DKKVEPKSCDKTHTCP**PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED**
PEVKFNWYVDGVEVHNA**TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN**
KALPAIEKTISKAKGQ**PREPVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN**
GQPENNYK**TPPVLDSDGSFFLYSKLTVDKSRWQQGNV**FSCSV**MHEALHNHYTQKSL**
SLSPGK (SEQ ID NO: 274)

Humanized 7F11 clone full-length light chain sequence

7F11-K2

MEAPAQLLFLLLLWLPDTTGDIVMTQTPLSLSVTPGQPASISCRSSQSL**VHTNGNSYLH**
WYLQKPGQSPQ**LLIYMVSNRFS**GV**PDRFSGSGSGTDFTLKISRVEAEDVGVYYC****SQSTH**
VPFTFGQGT**KL**EIKRTVAAPS**VIFPPSDEQLKSGTASVVC**LLNNFY**P**REAKVQ**W**KVDN
ALQSGNSQESVTEQDSKDSTYLS**SSLT**LSKADY**E**KKVYACEV**TH**QGLSSP**V**TKSFNR
GEC (SEQ ID NO: 275)

FIG. 107

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Humanized 9D9 clone full-length heavy chain sequences**9D9-H16**

MEAPAQLLFLLLLWLPDTTGEVQLLESGGGLVQPGGSLRLSCAASGFTFSDAWMDWV
 RQAPGKGLELVSEIRNKAKNHATYYAESVKGRFTISRDN SKNTLYLQMNSLRAEDTAV
 YYCTTLD^{SWGQ}GT^{TV}VSSASTKGPSVF^{FLAPSSK}STSGGTAALGCLVKDYFPEPVTVS
 WNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE
 PKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP^{EVTCVVVDVSHEDPEVKFN}
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNV^{FSCVMHEALHNHYTQKSLSLSPGK}
 (SEQ ID NO: 276)

9D9-H18

MEAPAQLLFLLLLWLPDTTGEVQLLESGGGLVQPGGSLRLSCAASGFTFSDAWMDWV
 RQAPGKGLELV^{AEIRNKAKNHATYYAESVKGRFTISRDN}SKNTLYLQMNSLRAEDTAV
 YYCTTLD^{SWGQ}GT^{TV}VSSASTKGPSVF^{FLAPSSK}STSGGTAALGCLVKDYFPEPVTVS
 WNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE
 PKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP^{EVTCVVVDVSHEDPEVKFN}
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNV^{FSCVMHEALHNHYTQKSLSLSPGK}
 (SEQ ID NO: 277)

Humanized 9D9 clone full-length light chain sequence**9D9-K13**

MEAPAQLLFLLLLWLPDTTGDIVMTQTPLSLSVTPGQPASISCKSSQSLDSDGKTYLNW
 LLQKPGQSPQRLIYLV^{SALDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC}WQGT^{HF}
 PWT^{FG}QGQTKLEIKRTVAAPS^{VFI}PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA
 LQSGNSQESVTEQDSKDYSLSSITLTSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
 EC (SEQ ID NO: 278)

FIG. 108

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Humanized 12G6 clone full-length heavy chain sequence**12G6-SFD1**

MEAPAQLLFLLLWLPDTTGEVQLVESGGGLVQPGGSLRLSCAASGFPFSNYWMN
WVRQAPGKGLEWVGQIRLKSNNYATHYAESVKGRFTISRDDSKNSLYLQMNSLKTE
DTAVYYCTPIDYWGQGTITVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNT
KVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNAKALPAIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNH
YTQKSLSLSPGK (SEQ ID NO: 279)

Humanized 12G6 clone full-length light chain sequence**12G6-K12**

MEAPAQLLFLLLWLPDTTGDIVMTQTPLSLSVTPGQPASISCKSSQSLLYSNGKTYL
NWVLQKPGQSPQRLIYLYSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQ
GSHFHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK
VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT
KSFNRGEC (SEQ ID NO: 280)

FIG. 109

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Humanized 4B10 clone full-length heavy chain sequence

4B10-H1

MEAPAQLLFLLLWLPDTTGEVQLVESGGGLVQPGGSLRLSCAASGFTFSDYYMSW
VRQAPGKGLEWVGFIRNKANGYTTEYSASVKGRFTISRDDSKNSLYLQMNSLKTED
TAVYYCARYIWFDYWGQGT^TTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYK^TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFC^SVMHEAL
HNHYTQKSLSLSPGK (SEQ ID NO: 281)

Humanized 4B10 clone full-length light chain sequence

4B10-K1

MEAPAQLLFLLLWLPDTTGDIVMTQTPLSLSVTPGQPASISCRSSQSLVHTNGNTYL
HWYLQKPGQSPQLLIYMVSNRFGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQ
SAHVPPLTFGQTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFFYPREAKVQ
WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSS
PVTKSFNRGEC (SEQ ID NO: 282)

FIG. 110

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Humanized 2C3 clone full-length heavy chain nucleic acid sequence**2C3-SFD1**

ATGGAAGCCCCAGCGCAGCTTCTCTTCCTCTGCTACTCTGGCTCCCTGATACCACCGGA
GAGGTACAGCTGGTGGAGTCGGGAGGAGGCTTGGTACAGCCTGGGGTTCTCTGA
GACTCTCCTGTGCAGCTTCTGGATTCACTTTCAATACCTACTGGATGAACTGGGTCC
GCCAGGCTCCAGGGAAGGACTTGAGTGGGTGGGTCAAATTAGATTGAAATCTAAT
AATTATGCAACACATTATGCGGAGTCTGTGAAAGGGCGGTTACCATCTCCAGAGA
TGATTCCAAAAACAGCCTCTATCTTCAAATGAATTCCCTGAAAACTGAAGACACTGC
CGTTTATTACTGTACCCCAAGTTGACTTTTGGGGCCAAGGCACCACTGTACAGTCTC
CTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCTCTCTCCAAGAGCACCTCTG
GGGGTACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTG
TGGAACTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCTACAGTCTCAGG
ACTTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT
CTGCACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAATCTTG
TGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACCTCTGGGGGGACCGTCAGTCTT
CCTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTACATGCGT
GGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCACTGGTACGTGGACGGCGTGG
AGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
AGCGTCTCACCGTCTGCACCAAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCC
AACAAGCCCTCCAGCCCCATCGAGAAAACCTCTCCAAGCCAAAGGGCAGCCCCGAGAA
CCACAGGTGTACACCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGAC
ATGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGC
CGGAGAACAACTACAAGACCACGCTCCCGTGTGGACTCCGACGGCTCTTCTCTCTACA
GCAAGCTCACCGTGGACAAGTCCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG
CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCTGTCTCCGGGTAAATGA
(SEQ ID NO: 283)

Humanized 2C3 clone full-length light chain nucleic acid sequence**2C3-K12**

ATGGAAGCCCCAGCGCAGCTTCTCTTCCTCTGCTACTCTGGCTCCCTGATACCACCGGA
GACATTGTGATGACCCAGACTCCACTCAGTTTGTCAAGTTACCCCTGGACAACCAGC
CTCAATCTCTTGCAAGTCAAGTCAGAGCCTCTTATATAGTAATGGAAAAACCTATTT
GAACTGGTTATTACAGAAGCCAGGCCAGTCTCCACAGCGCCTAATCTATCTGGTGT
CTAAATTGGACTCTGGAGTCCCTGACAGGTTCAAGTGGCAGTGGATCAGGAACAGAT
TTTACACTGAAAATCAGCAGAGTGGAGGCTGAGGATGTGGGAGTTTATTACTCGCT
GCAAGGTACACATCTGCACACGTTCCGTCAAGGGACCAAGGCTGGAGATAAAACGAA
CTGTGGCAGCACCAAGCGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAC
CTCTGTTGTGTGCCTGTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGAAGGTGGAT
AACGCCCTCCAAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGC
CTACAGCCTCAGCAGCACCTGACGCTGAGCAAGCAGACTACGAGAAACAAAGTCTACGC
CTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGT
GTTAG (SEQ ID NO: 284)

FIG. 111

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Humanized 7F11 clone full-length heavy chain nucleic acid sequence**7F11-SFD1**

ATGGAAGCCCCAGCGCAGCTTCTCTCCTCTGCTACTCTGGCTCCCTGATACCACCGGA
GAGGTACAGCTGGTGGAGTCGGGAGGAGGCTTGGTACAGCCTGGGGGTTCTCTGA
GACTCTCCTGTGCAGCTTCTGGCTTCACATTCACCGACTATTACATGAGCTGGGTCC
GCCAGGCTCCAGGGAAGGACTTGAGTGGGTGGGTTTCATAAGGAACAAGGCTAA
CGGTTATACAACCGAGTACAACGCTTCCGTTAAAGGCCGGTTACCATCTCCAGAG
ATGATTCCAAAAACAGCCTCTATCTTCAAAATGAATTCCTGAAAACTGAAGACACTG
CCGTTTATTACTGTACCAGGTATATCTTTTCGATTACTGGGGCCAAGGCACCACTG
TCACAGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCTGGCACCTCTCTCAA
GAGCACCTCTGGGGGTACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGG
TGACGGGTGTCGTGGAACCTAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGTGTCTTA
CAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCTCCAGCAGCTTGGGCACC
CAGACCTACATCTGCAACGTGAATCACAAGCCAGCAACACCAAGGTGGACAAGAAAGTTGAG
CCCAAATCTTGTGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACCTCTGGGGGGA
CCGTGAGTCTTCTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCCGGACCCCTGAG
GTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTG
GACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTA
CCGTGTGGTCAGCGTCTCACCGTCTGCACCAAGGACTGGCTGAATGGCAAGGAGTACAAGT
GCAAGGTCTCAACAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAAGCCAAGGGC
AGCCCCGAGAACACAGGTGTACACCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCCAG
GTCAGCCTGACATGCCTGGTCAAGGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAG
CAATGGGCAGCCGGAGAACAACTACAAGACCACGCCCTCCCGTGTGGACTCCGACGGCTCCT
TCTTCTCTACAGCAAGCTCACCGTGGACAAGTCCAGGTGGCAGCAGGGGAACGTCTTCTCAT
GCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGG
GTAAATGA (SEQ ID NO: 285)

Humanized 7F11 clone full-length light chain nucleic acid sequence**7F11-K2**

ATGGAAGCCCCAGCGCAGCTTCTCTCCTCTGCTACTCTGGCTCCCTGATACCACCGGA
GATATTGTAATGACCCAAACACCCCTCTCTTTTCACTACACCTGGACAGCCAGCG
TCCATCTCCTGCAGGTCCTCACAGAGTCTCGTGCACACCAATGGCAATTCCTACCTG
CATTGGTACCTGCAGAAGCCCGGGCAGAGCCCCAGTTGCTGATCTATATGGTGTG
TAATCGGTTCTCCGGAGTCCCGACAGATTTTCTGGTTCAGGGTCTGGAACCTGATTT
TACACTGAAGATTAGTCGGGTTCGAGGCCGAGGATGTAGGCGGTGATTACTGCTCAC
AAAGCACACATGTGCCGTTCACTTTCCGCCAAGGAACAAAGCTCGAAATCAAGCGAA
CTGTGGCAGCACCAAGCGTCTTCACTTCCCAGCATCTGATGAGCAGTTGAAATCTGGAACCTG
CTCTGTTGTGTGCTGTGAATACTTCTATCCCAGAGAGGCCAAAGTACAGTGAAGGTGGAT
AACGCCCTCCAATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCAC
CTACAGCCTCAGCAGCACCTGACGCTGAGCAAGCAGACTACGAGAAACAAAGTCTACGC
CTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGT
GTTAG (SEQ ID NO: 286)

FIG. 112

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Humanized 9D9 clone full-length heavy chain nucleic acid sequences**9D9-H16**

ATGGAAGCCCCAGCGCAGCTTCTCTCTCTCTGCTACTCTGGCTCCCTGATACCAACGGAGAG
GTACAGCTTCTTGAAAGTGGAGGTGGCCTTGTTCCAACCCGGAGGGTCAATTGCGGTGAG
CTGTGCGGCAAGTGGCTTCACCTTCTCTGACGCTTGGATGGACTGGGTGAGACAAGCCC
CCGGTAAGGGACTGGAGTTGGTTTCTGAAATCAGGAACAAGGCCAAGAACCATGCAAC
ATATTATGCCGAAAGTGTGAAGGGAAGGTTACAATCAGTAGAGATAACAGCAAGAACA
CACTGTACCTCCAGATGAACAGCCTCAGAGCTGAGGACACCGCCGTCTATTATTGTACC
ACTCTCGATTTCATGGGGGCAGGGTACCACCGTTACAGTCAGCAGCGCTCCACCAAGGGC
CCATCGGTCTTCCCCCTGGCACCCCTCTCCAAGAGCACCTCTGGGGGTACAGCGGCCCTGGGCTG
CCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACACGG
GCGTGCACACCTTCCCCGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCG
TGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCA
AGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCCGTGCCAGCAC
CTGAATCTCTGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCAAGGACACCTCATGATCT
CCCCGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTC
AATGTTGACGTGGACCGGCTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAA
CAGCACGTACCGTGTGGTACGCTCTCACCCTCTGCACCAAGGACTGGCTGAATGGCAAGGAGT
ACAAGTGCAAGGTCTCCAACAAGCCCTCCAGCCCCATCGAGAAAACCATCTCCAAGCCAAAG
GGCAGCCCCGAGAACCAAGGTGTACACCTGCCCCATCCCGGGATGAGCTGACCAAGAACCAG
GTCAGCCTGACATGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT
GGGCAGCCGGAGAACAACTACAGACCAGCCTCCCGTCTGGACTCCGACGGCTCCTTCTCTCTC
TACAGCAAGCTCACCGTGGACAAGTCCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG
CATGAGGCTCTGCACAACCACTACACGCAGAAAGACCTCTCCCTGTCTCCGGGTAATGA (SEQ
ID NO: 287)

9D9-H18

ATGGAAGCCCCAGCGCAGCTTCTCTCTCTCTGCTACTCTGGCTCCCTGATACCAACGGAGAG
GTACAGCTTCTTGAAAGTGGAGGTGGCCTTGTTCCAACCCGGAGGGTCAATTGCGGTGAG
CTGTGCGGCAAGTGGCTTCACCTTCTCTGACGCTTGGATGGACTGGGTGAGACAAGCCC
CCGGTAAGGGACTGGAGTTGGTTGCTGAAATCAGGAACAAGGCCAAGAACCATGCAAC
ATATTATGCCGAAAGTGTGAAGGGAAGGTTACAATCAGTAGAGATAACAGCAAGAACA
CACTGTACCTCCAGATGAACAGCCTCAGAGCTGAGGACACCGCCGTCTATTATTGTACC
ACTCTCGATTTCATGGGGGCAGGGTACCACCGTTACAGTCAGCAGCGCTCCACCAAGGGC
CCATCGGTCTTCCCCCTGGCACCCCTCTCCAAGAGCACCTCTGGGGGTACAGCGGCCCTGGGCTG
CCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACACGG
GCGTGCACACCTTCCCCGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCG
TGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCA
AGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCCGTGCCAGCAC
CTGAATCTCTGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCAAGGACACCTCATGATCT
CCCCGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTC
AATGTTGACGTGGACGGCTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAA
CAGCACGTACCGTGTGGTACGCTCTCACCCTCTGCACCAAGGACTGGCTGAATGGCAAGGAGT
ACAAGTGCAAGGTCTCCAACAAGCCCTCCAGCCCCATCGAGAAAACCATCTCCAAGCCAAAG
GGCAGCCCCGAGAACCAAGGTGTACACCTGCCCCATCCCGGGATGAGCTGACCAAGAACCAG
GTCAGCCTGACATGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT
GGGCAGCCGGAGAACAACTACAGACCAGCCTCCCGTCTGGACTCCGACGGCTCCTTCTCTCTC
TACAGCAAGCTCACCGTGGACAAGTCCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG
CATGAGGCTCTGCACAACCACTACACGCAGAAAGACCTCTCCCTGTCTCCGGGTAATGA (SEQ
ID NO: 288)

FIG. 113

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Humanized 9D9 clone full-length light chain nucleic acid sequence**9D9-K13**

ATGGAAGCCCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCCCTGATACCACCGGA
GATATCGTGATGACACAACTCCCCTGTCTCTGTCTGTAAGTCCAGGTCAGCCCGC
GAGTATTTTCATGTAAGAGCAGCCAATCCCTGCTGGACAGCGACGGGAAGACCTACC
TGAAGTGGTTACTCCAAAAGCCAGGACAAAGTCCCAACGCCTTATTTACCTGGTGT
CAGCCCTGGACTCTGGCGTGCCCGATCGATTTAGCGGCAGCGGGAGTGGCACAGAT
TTCACCCTGAAAATATCCCGCGTCGAGGCCGAAGATGTGGGCGTGTACTACTGCTG
GCAGGGGCACACATTTCCCTGGACATTTGGTCAGGGGACAAAGCTGGAAATTAAC
GAAGTGTGGCAGCACCAAGCGTCTTCATCTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAC
TGCCCTCTGTTGTGTGCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAAGGTG
GATAACGCCCTCCAATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAG
CACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTA
CGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAG
AGTGTTAG (SEQ ID NO: 289)

FIG. 114

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Humanized 12G6 clone full-length heavy chain nucleic acid sequence**12G6-SFD1**

ATGGAAGCCCCAGCGCAGCTTCTCTTCTCTGCTACTCTGGCTCCCTGATACCACCGGA
GAGGTACAGCTGGTGGAGTCGGGAGGAGGCTTGGTACAGCCTGGGGTTCTCTGA
GACTCTCTCTGTGAGCTTCTGGATTCCCATTCAGTAACACTACTGGATGAACTGGGTCC
GCCAGGCTCCAGGGAAGGACTTGAGTGGGTGGGTCAAATTAGATTGAAATCTAAT
AATTATGCAACACATTATGCGGAGTCTGTGAAAGGGCGGTTACCATCTCCAGAGA
TGATTCCAAAAACAGCCTCTATCTTCAAATGAATTCCCTGAAAACTGAAGACACTGC
CGTTTATTACTGTACCCCAATTGACTATTGGGGCCAAGGCACCACTGTACAGTCTC
CTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCTGGCACCTCTCTCAAGAGCACCTCTG
GGGGTACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTG
TGGAACTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCTACAGTCTCAGG
ACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT
CTGCACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAATCTTG
TGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACCTCTGGGGGGACCGTCAGTCTT
CCTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTACATGCGT
GGTGGTGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG
AGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
AGCGTCTCACCCTCTGCACCAAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCC
AACAAAGCCCTCCAGCCCCATCGAGAAAACCTCTCAAAGCCAAAGGGCAGCCCCGAGAA
ACACAGGTGTACCCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAAGTACGCTGAC
ATGCTGGTCAAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGC
CGGAGAACAACTACAAGACCACGCTCCCGTGCTGGACTCCGACGGCTCTTCTCTCTACA
GCAAGCTCACCGTGGACAAGTCCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG
CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA
(SEQ ID NO: 290)

Humanized 12G6 clone full-length light chain nucleic acid sequence**12G6-K12**

ATGGAAGCCCCAGCGCAGCTTCTCTTCTCTGCTACTCTGGCTCCCTGATACCACCGGA
GACATTGTGATGACCCAGACTCCACTCAGTTTGTCTAGTTACCCCTGGGCAACCAGC
CTCTATCTCTTGCAAGTCAAGTCAGAGCCTCTTATATAGTAATGGAAAAACCTATTT
GAACTGGGTTTTACAGAAGCCAGGCCAGTCTCCACAGCGCCTAATCTATCTGGTGT
CTAACTGGACTCTGGAGTCCCTGACAGGTTCTCTGGCAGTGGATCAGGAACAGAT
TTTACACTGAAAATCAGCAGAGTGGAGGCTGAGGATGTGGGAGTTTATTACTGCGT
GCAAGGTTACATTTTACACGTTCCGGTCAAGGGACCAAGCTGGAGATTAACGAAC
TGTGGCAGCACCAAGCGTCTTCTATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGC
TCTGTTGTGCTGCTGAATAACTTCTATCCAGAGAGGCCAAAGTACAGTGGAAAGGTGGATA
ACGCCCTCCAATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACC
TACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCC
TGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTG
TTAG (SEQ ID NO: 291)

FIG. 115

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Humanized 4B10 clone full-length heavy chain nucleic acid sequence**4B10-H1**

ATGGAAGCCCCAGCGCAGCTTCTCTTCCTCTGCTACTCTGGCTCCCTGATACCACCGGA
GAGGTACAGCTGGTGGAGTCGGGAGGAGGCTTGGTACAGCCTGGGGGTTCTCTGA
GACTCTCCTGTGCAGCTTCTGGATTACCTTTTCTGATTACTACATGAGCTGGGTCC
GCCAGGCTCCAGGGAAGGACTTGAGTGGGTGGGTTTTATTAGAAACAAAGCTAAT
GGTTACACAACAGAGTACAGTGCATCTGTGAAGGGTCGGTTACCATCTCCAGAGA
TGATTCCAAAAACAGCCTCTATCTTCAAATGAATTCCCTGAAAACTGAAGACACTGC
CGTTTATTACTGTGCAAGATATATCTGGTTTGACTACTGGGGCCAAGGCACCACTGT
CACAGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCTGGCACCTCTCTCAAG
AGCACCTCTGGGGGTACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCGAACC
GACGGTGTCTGGAACTCAGGCGCCCTGACCAGCGCGTGCACACTTCCCGGTGTCTTAC
AGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCTCCAGCAGCTTGGGCACCC
AGACTACATCTGCAACGTGAATCACAAGCCAGCAACACCAAGGTGGACAAGAAAGTTGAGC
CCAAATCTTGTGACAAAACACACATGCCACCCGTGCCAGCAGCTGAATCCTGGGGGGAC
CGTCAGTCTTCTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCCGGACCCCTGAGG
TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGG
ACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTAC
CGTGTGGTCAGCGTCTCACCCTCTGCACCAAGGACTGGCTGAATGGCAAGGAGTACAAGTG
CAAGGTCTCCAACAAGCCCTCCAGCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCA
GCCCCGAGAACACAGGTGTACACCTGCCCCCATCCCGGATGAGCTGACCAAGAACCAAGG
TCAGCCTGACATGCTGGTCAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCA
ATGGGACGCCGAGAACAACTACAAGACCAAGCCTCCCGTGTGGACTCCGACGGCTCTCTCT
TCCTCTACAGCAAGCTCACCGTGGACAAGTCCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT
CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGA
AATGA (SEQ ID NO: 292)

Humanized 4B10 clone full-length light chain nucleic acid sequence**4B10-K1**

ATGGAAGCCCCAGCGCAGCTTCTCTTCCTCTGCTACTCTGGCTCCCTGATACCACCGGA
GACATTGTGATGACCCAACTCCACTCTCCCTGTCTGTCACTCCTGGACAACCAAGCC
TCCATCTCTTGCAGATCTAGTCAGAGCCTTGTACACACTAATGGAAACACCTATTTA
CATTGGTACCTGCAGAAGCCAGGCCAGTCTCCACAGCTCCTGATTTATATGGTTTCC
AACCATTCTTGGGGTCCCAGACAGGTTCAAGTGGCAGTGGATCAGGGACAGATTT
CACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTGGGAGTTTATTACTGCTCTC
AAAGTGACATGTTCTCCGCTCACGTTCCGTTCAAGGACCAGGCTGGAGATTA
CGAATCTGGGACGACCAAGCGTCTTCACTTCCCGCATCTGATGAGCAGTTGAAATCTGGAA
CTGCCTCTGTTGTGTGCTGTGAATACTTCTATCCAGAGAGGCCAAAGTACAGTGGAGGT
GGATAACGCCCTCCAATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACA
GCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAGCAGACTACGAGAAACAAAGTCT
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GAGTGTAG (SEQ ID NO: 293)

FIG. 116

Sequence Listing
SEQUENCE LISTING

<110> GENZYME CORPORATION

<120> ANTI-HUMAN CD52 IMMUNOGLOBULINS

<130> 001662-0029-W01

<140> PCT/US2010/034704

<141> 2010-05-13

<150> 61/177,837

<151> 2009-05-13

<160> 294

<170> PatentIn version 3.5

<210> 1

<211> 107

<212> PRT

<213> Rattus sp.

<400> 1

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

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

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

Tyr Asn Thr Asn Asn Leu Gln Thr Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Val Ala Thr Tyr Phe Cys Leu Gln His Ile Ser Arg Pro Arg
85 90 95

Thr Phe Gly Thr Gly Thr Lys Leu Glu Leu Lys
100 105

<210> 2

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 2

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

His Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Glu Ser
20 25 30

Sequence Listing

Asp Gly Arg Thr Tyr Leu Asn Trp Leu Phe Gln Arg Pro Gly Gln Ser
35 40 45

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

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

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

Thr His Phe Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu Lys
100 105 110

<210> 3
<211> 112
<212> PRT
<213> Mus sp.

<400> 3
Asp Ile Val Leu Thr Gln Ser Thr Leu Ser Leu Ser Val Thr Ile Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30

Asp Gly Lys Thr Tyr Leu Asn Trp Met Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

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

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

Thr His Phe Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 4
<211> 112
<212> PRT
<213> Mus sp.

<400> 4
Asp Ile Val Ile Thr Gln Ser Thr Leu Thr Leu Ser Val Thr Ile Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30

Sequence Listing

Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Lys Arg Leu Met Tyr Leu Val Ser Asn Leu Gly Ser Gly Val Pro
50 55 60

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

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

Thr His Phe Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Ile
100 105 110

<210> 5
<211> 112
<212> PRT
<213> Mus sp.

<400> 5
Asp Ile Val Met Thr Gln Thr Pro Leu Thr Leu Ser Val Thr Ile Gly
1 5 10 15

Gln Pro Ala Ser Ile Phe Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30

Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro Gly Gln Ser
35 40 45

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

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

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

Thr His Phe Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 6
<211> 112
<212> PRT
<213> Mus sp.

<400> 6
Asp Ile Val Met Thr Gln Thr Pro Leu Thr Leu Ser Val Thr Ile Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30

Sequence Listing

Asp Gly Lys Thr Tyr Leu Asn Trp Leu Ser Gln Arg Pro Gly Gln Ser
35 40 45

Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

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

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

Thr His Phe Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 7
<211> 112
<212> PRT
<213> Mus sp.

<400> 7
Asp Ile Val Met Thr Gln Ser Pro Leu Thr Leu Ser Val Thr Ile Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30

Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Lys Arg Leu Ile Tyr Leu Val Ser Asn Leu Asn Ser Gly Leu Pro
50 55 60

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

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

Thr His Phe Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 8
<211> 112
<212> PRT
<213> Mus sp.

<400> 8
Asp Ile Val Leu Thr Gln Thr Thr Leu Thr Leu Ser Val Thr Ile Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30

Sequence Listing

Asp Gly Arg Thr Tyr Leu Asn Trp Leu Phe Gln Arg Pro Gly Gln Ser
35 40 45

Pro Lys Arg Leu Ile Phe Leu Val Ser His Leu Asp Ser Gly Val Pro
50 55 60

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

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

Thr His Phe Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 9
<211> 111
<212> PRT
<213> Mus sp.

<400> 9
Asp Ile Val Met Thr Gln Thr Pro Leu Thr Leu Ser Val Thr Ile Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

Asn Gly Lys Thr Tyr Leu Asn Trp Val Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

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

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

Ser His Phe His Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105 110

<210> 10
<211> 111
<212> PRT
<213> Mus sp.

<400> 10
Asp Ile Val Leu Thr Gln Thr Pro Arg Thr Leu Ser Val Thr Ile Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

Sequence Listing

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

Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

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

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

Thr Arg Phe His Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105 110

<210> 11
<211> 111
<212> PRT
<213> Mus sp.

<400> 11
Asp Ile Val Ile Thr Gln Thr Pro Leu Thr Leu Ser Val Thr Ile Gly
1 5 10 15

Gln Pro Val Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

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

Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

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

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

Thr His Leu His Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105 110

<210> 12
<211> 112
<212> PRT
<213> Mus sp.

<400> 12
Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Thr
20 25 30

Sequence Listing

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

Pro Lys Leu Leu Ile Tyr Met Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60

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

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

Thr His Val Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
 100 105 110

<210> 13
 <211> 113
 <212> PRT
 <213> Mus sp.

<400> 13
 Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Thr Val Ser Leu Gly
 1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Thr
 20 25 30

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

Pro Lys Leu Leu Ile Tyr Met Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60

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

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

Ala His Val Pro Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
 100 105 110

Lys

<210> 14
 <211> 121
 <212> PRT
 <213> Rattus sp.

<400> 14
 Glu Val Lys Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Sequence Listing

Ser Met Arg Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Thr Asp Phe
 20 25 30

Tyr Met Asn Trp Ile Arg Gln Pro Ala Gly Lys Ala Pro Glu Trp Leu
 35 40 45

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

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Thr Gln Asn Met
 65 70 75 80

Leu Tyr Leu Gln Met Asn Thr Leu Arg Ala Glu Asp Thr Ala Thr Tyr
 85 90 95

Tyr Cys Ala Arg Glu Gly His Thr Ala Ala Pro Phe Asp Tyr Trp Gly
 100 105 110

Gln Gly Val Met Val Thr Val Ser Ser
 115 120

<210> 15
 <211> 114
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic polypeptide

<400> 15
 Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Met Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ala
 20 25 30

Trp Met Asp Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Val
 35 40 45

Ala Glu Ile Arg Asn Lys Ala Lys Asn His Val Ala Tyr Tyr Ala Glu
 50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ser
 65 70 75 80

Val Tyr Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr Gly Ile Tyr
 85 90 95

Tyr Cys Thr Thr Leu Asp Ser Trp Gly Gln Gly Thr Ala Leu Thr Val
 100 105 110

Sequence Listing

Ser Ser

<210> 16
<211> 114
<212> PRT
<213> Mus sp.

<400> 16
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Met Lys Leu Ser Cys Ala Val Ser Arg Phe Thr Phe Ser Asp Ala
20 25 30

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

Ala Glu Ile Arg Asn Lys Ala Asn Asn His Ala Thr Tyr Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Arg
65 70 75 80

Val Phe Leu Gln Met Asn Asn Leu Arg Pro Glu Asp Thr Gly Ile Tyr
85 90 95

Tyr Cys Thr Ser Leu Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val
100 105 110

Ser Ser

<210> 17
<211> 114
<212> PRT
<213> Mus sp.

<400> 17
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Met Lys Leu Ser Cys Ala Val Ser Gly Phe Thr Phe Ser Asp Ala
20 25 30

Trp Met Asp Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Val
35 40 45

Ala Glu Ile Arg Asn Lys Ala Lys Asn His Val Lys Tyr Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ser
65 70 75 80

Sequence Listing

Val Tyr Leu Gln Met Asn Asn Leu Arg Thr Glu Asp Thr Gly Ile Tyr
85 90 95

Tyr Cys Thr Ser Leu Asp Tyr Trp Gly Gln Gly Thr Ala Leu Thr Val
100 105 110

Ser Ser

<210> 18
<211> 114
<212> PRT
<213> Mus sp.

<400> 18
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Met Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ala
20 25 30

Trp Met Asp Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Leu Thr
35 40 45

Ala Glu Ile Arg Asn Lys Ala Lys Asn His Ala Thr Tyr Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Arg
65 70 75 80

Val Tyr Leu Gln Met Asn Ser Leu Arg Thr Glu Asp Thr Gly Ile Tyr
85 90 95

Tyr Cys Thr Thr Leu Asp Ser Trp Gly Gln Gly Thr Ser Val Thr Val
100 105 110

Ser Ser

<210> 19
<211> 114
<212> PRT
<213> Mus sp.

<400> 19
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Met Lys Leu Ser Cys Ala Val Ser Gly Phe Thr Phe Ser Asp Ala
20 25 30

Trp Met Asp Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Val
35 40 45

Sequence Listing

Ala Glu Ile Arg Asn Lys Ala Lys Asn His Ala Thr Tyr Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ser
65 70 75 80

Val Tyr Leu Gln Met Asn Arg Leu Arg Ala Glu Asp Thr Gly Ile Tyr
85 90 95

Tyr Cys Thr Ser Leu Asp Tyr Trp Gly Gln Gly Ser Thr Leu Thr Val
100 105 110

Ser Ser

<210> 20
<211> 114
<212> PRT
<213> Mus sp.

<400> 20
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Met Lys Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ser Asp Ala
20 25 30

Trp Met Asp Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Val
35 40 45

Ala Glu Ile Arg Lys Lys Val Asn Asn His Ala Thr Tyr Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ser
65 70 75 80

Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Gly Ile Tyr
85 90 95

Tyr Cys Thr Ser Leu Asp Tyr Trp Gly Gln Gly Thr Thr Leu Ser Val
100 105 110

Ser Ser

<210> 21
<211> 114
<212> PRT
<213> Mus sp.

<400> 21
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Sequence Listing

Ser Met Lys Leu Ser Cys Ala Val Ser Gly Leu Thr Phe Ser Asp Ala
20 25 30

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

Ala Glu Ile Arg Asn Lys Ala Lys Asn His Ala Thr Tyr Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Gly
65 70 75 80

Val Tyr Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr Gly Ile Tyr
85 90 95

Tyr Cys Thr Gly Leu Asp Tyr Trp Gly His Gly Thr Ser Val Thr Val
100 105 110

Ser Ser

<210> 22
<211> 114
<212> PRT
<213> Mus sp.

<400> 22
Glu Val Gln Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Met Lys Leu Ser Cys Val Ala Ser Gly Phe Pro Phe Ser Asn Tyr
20 25 30

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

Ala Gln Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr His Tyr Ala Glu
50 55 60

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

Val Tyr Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr Gly Ile Tyr
85 90 95

Tyr Cys Thr Pro Ile Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val
100 105 110

Ser Ser

<210> 23
<211> 114

Sequence Listing

<212> PRT

<213> Mus sp.

<400> 23

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

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

Trp Met Asn Trp Val Arg Gln Ser Pro Asp Lys Gly Leu Glu Cys Ile
35 40 45

Ala Gln Ile Arg Leu Lys Ser Asp Asn Tyr Ala Thr His Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ser
65 70 75 80

Val Tyr Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr Gly Ile Tyr
85 90 95

Tyr Cys Thr Pro Ile Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val
100 105 110

Ser Ser

<210> 24

<211> 114

<212> PRT

<213> Mus sp.

<400> 24

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

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

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

Ala Gln Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr His Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
65 70 75 80

Val Tyr Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr Gly Ile Tyr
85 90 95

Tyr Cys Thr Pro Val Asp Phe Trp Gly Gln Gly Thr Thr Leu Thr Val
100 105 110

Sequence Listing

Ser Ser

<210> 25
 <211> 117
 <212> PRT
 <213> Mus sp.

<400> 25
 Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Ser Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Thr Asp Tyr
 20 25 30

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

Gly Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Asn Ala
 50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Tyr Ser Gln Ser Ile
 65 70 75 80

Leu Tyr Leu Gln Met Asn Ala Leu Arg Ala Glu Asp Ser Ala Thr Tyr
 85 90 95

Tyr Cys Thr Arg Tyr Ile Phe Phe Asp Tyr Trp Gly Gln Gly Thr Thr
 100 105 110

Leu Thr Val Ser Ser
 115

<210> 26
 <211> 117
 <212> PRT
 <213> Mus sp.

<400> 26
 Glu Val Gln Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ala Leu Ser Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Thr Asp Tyr
 20 25 30

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

Gly Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Ser Ala
 50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Gln Ser Ile
 65 70 75 80

Sequence Listing

Leu Tyr Leu Gln Met Asn Ala Leu Arg Ala Glu Asp Ser Ala Thr Tyr
 85 90 95

Tyr Cys Thr Arg Tyr Ile Trp Phe Asp Tyr Trp Gly Gln Gly Thr Thr
 100 105 110

Leu Thr Val Ser Ser
 115

<210> 27
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 27
 Lys Ala Ser Gln Asn Ile Asp Lys Tyr Leu Asn
 1 5 10

<210> 28
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 28
 Lys Ser Ser Gln Ser Leu Leu Glu Ser Asp Gly Arg Thr Tyr Leu Asn
 1 5 10 15

<210> 29
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 29
 Lys Ser Ser Gln Ser Leu Leu Asp Ser Asp Gly Lys Thr Tyr Leu Asn
 1 5 10 15

<210> 30
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 30
 Lys Ser Ser Gln Ser Leu Leu Asp Ser Asp Gly Arg Thr Tyr Leu Asn
 1 5 10 15

Sequence Listing

<210> 31
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 31
Lys Ser Ser Gln Ser Leu Leu Tyr Ser Asn Gly Lys Thr Tyr Leu Asn
1 5 10 15

<210> 32
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 32
Arg Ser Ser Gln Ser Leu Val His Thr Asn Gly Asn Ser Tyr Leu His
1 5 10 15

<210> 33
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 33
Arg Ser Ser Gln Ser Leu Val His Thr Asn Gly Asn Thr Tyr Leu His
1 5 10 15

<210> 34
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 34
Asn Thr Asn Asn Leu Gln Thr
1 5

<210> 35
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 35

Sequence Listing

Leu Val Ser Asn Leu Asp Ser
1 5

<210> 36
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 36
Leu Val Ser Lys Leu Asp Ser
1 5

<210> 37
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 37
Leu Val Ser Asn Leu Gly Ser
1 5

<210> 38
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 38
Leu Val Ser Ala Leu Asp Ser
1 5

<210> 39
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 39
Leu Val Ser Asn Leu Asn Ser
1 5

<210> 40
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

Sequence Listing

<400> 40
Leu Val Ser His Leu Asp Ser
1 5

<210> 41
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 41
Met Val Ser Asn Arg Phe Ser
1 5

<210> 42
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 42
Leu Gln His Ile Ser Arg Pro Arg Thr
1 5

<210> 43
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 43
Trp Gln Gly Thr His Phe Pro Trp Thr
1 5

<210> 44
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 44
Val Gln Gly Ser His Phe His Thr
1 5

<210> 45
<211> 8
<212> PRT
<213> Artificial Sequence

<220>

Sequence Listing

<223> Description of Artificial Sequence: Synthetic peptide

<400> 45

Val Gln Gly Thr Arg Phe His Thr
1 5

<210> 46

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 46

Val Gln Gly Thr His Leu His Thr
1 5

<210> 47

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 47

Ser Gln Ser Thr His Val Pro Phe Thr
1 5

<210> 48

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 48

Ser Gln Ser Ala His Val Pro Pro Leu Thr
1 5 10

<210> 49

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 49

Gly Phe Thr Phe Thr Asp Phe Tyr Met Asn
1 5 10

<210> 50

<211> 10

<212> PRT

<213> Artificial Sequence

Sequence Listing

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 50

Gly Phe Thr Phe Ser Asp Ala Trp Met Asp
1 5 10

<210> 51

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 51

Arg Phe Thr Phe Ser Asp Ala Trp Met Asp
1 5 10

<210> 52

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 52

Gly Leu Thr Phe Ser Asp Ala Trp Met Asp
1 5 10

<210> 53

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 53

Gly Phe Pro Phe Ser Asn Tyr Trp Met Asn
1 5 10

<210> 54

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 54

Gly Phe Thr Phe Asn Lys Tyr Trp Met Asn
1 5 10

<210> 55

<211> 10

Sequence Listing

<212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 55
 Gly Phe Thr Phe Asn Thr Tyr Trp Met Asn
 1 5 10

<210> 56
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 56
 Gly Phe Thr Phe Thr Asp Tyr Tyr Met Ser
 1 5 10

<210> 57
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 57
 Phe Ile Arg Asp Lys Ala Lys Gly Tyr Thr Thr Glu Tyr Asn Pro Ser
 1 5 10 15

Val Lys Gly

<210> 58
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 58
 Glu Ile Arg Asn Lys Ala Lys Asn His Val Ala Tyr Tyr Ala Glu Ser
 1 5 10 15

Val Lys Gly

<210> 59
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>

Sequence Listing

<223> Description of Artificial Sequence: Synthetic peptide

<400> 59

Glu Ile Arg Asn Lys Ala Asn Asn His Ala Thr Tyr Tyr Ala Glu Ser
1 5 10 15

Val Lys Gly

<210> 60

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 60

Glu Ile Arg Asn Lys Ala Lys Asn His Val Lys Tyr Tyr Ala Glu Ser
1 5 10 15

Val Lys Gly

<210> 61

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 61

Glu Ile Arg Asn Lys Ala Lys Asn His Ala Thr Tyr Tyr Ala Glu Ser
1 5 10 15

Val Lys Gly

<210> 62

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 62

Glu Ile Arg Lys Lys Val Asn Asn His Ala Thr Tyr Tyr Ala Glu Ser
1 5 10 15

Val Lys Gly

<210> 63

<211> 19

Sequence Listing

<212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 63
 Gln Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr His Tyr Ala Glu Ser
 1 5 10 15

Val Lys Gly

<210> 64
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 64
 Gln Ile Arg Leu Lys Ser Asp Asn Tyr Ala Thr His Tyr Ala Glu Ser
 1 5 10 15

Val Lys Gly

<210> 65
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 65
 Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Asn Ala Ser
 1 5 10 15

Val Lys Gly

<210> 66
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 66
 Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Ser Ala Ser
 1 5 10 15

Val Lys Gly

Sequence Listing

<210> 67
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 67
Ala Arg Glu Gly His Thr Ala Ala Pro Phe Asp Tyr
1 5 10

<210> 68
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 68
Thr Thr Leu Asp Ser
1 5

<210> 69
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 69
Thr Ser Leu Asp Tyr
1 5

<210> 70
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 70
Thr Gly Leu Asp Tyr
1 5

<210> 71
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 71

Sequence Listing

Thr Pro Ile Asp Tyr
1 5

<210> 72
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 72
Thr Pro Val Asp Phe
1 5

<210> 73
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 73
Thr Arg Tyr Ile Phe Phe Asp Tyr
1 5

<210> 74
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 74
Thr Arg Tyr Ile Trp Phe Asp Tyr
1 5

<210> 75
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic primer

<400> 75
atgggcwtca aratgrarwc wcat

24

<210> 76
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic primer

Sequence Listing

<400> 76
 gayattgtgm tracmcarkm tcaa 24

<210> 77
 <211> 21
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic primer

<400> 77
 actggatggt gggaagatgg a 21

<210> 78
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic primer

<400> 78
 gayattgtgm tsacmcarwc tmca 24

<210> 79
 <211> 21
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic primer

<400> 79
 ggatacagtt ggtgcagcat c 21

<210> 80
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic primer

<400> 80
 atgrasttsk ggytmarctk grtt 24

<210> 81
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic primer

<400> 81
 atgraatgsa sctgggtywt yctct 25

Sequence Listing

<210> 82
 <211> 21
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic primer

 <400> 82
 saggtsmarc tgcagsagtc t 21

<210> 83
 <211> 24
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic primer

 <400> 83
 saggtgmagc tcswrsaryc sggg 24

<210> 84
 <211> 24
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic primer

 <400> 84
 tgaggagact gtgagagtgg tgcc 24

<210> 85
 <211> 30
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic primer

 <400> 85
 ayctccacac acaggrcca gtggatagac 30

<210> 86
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic primer

 <220>
 <221> modified_base
 <222> (6)..(6)
 <223> a, c, t, g, unknown or other

Sequence Listing

<400> 86
sargtnmagc tgsagsagtc 20

<210> 87
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic primer

<220>
<221> modified_base
<222> (6)..(6)
<223> a, c, t, g, unknown or other

<400> 87
sargtnmagc tgsagsagtc wgg 23

<210> 88
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic primer

<400> 88
caggttactc tgaaagwgts tg 22

<210> 89
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic primer

<400> 89
gaggtccarc tgcaacartc 20

<210> 90
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic primer

<400> 90
caggtccaac tvcagcarcc 20

<210> 91
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

	Sequence Listing	
<223> Description of Artificial Sequence: Synthetic primer		
<400> 91 gaggtgaass tgggtggaatc		20
<210> 92 <211> 20 <212> DNA <213> Artificial Sequence		
<220> <223> Description of Artificial Sequence: Synthetic primer		
<400> 92 gatgtgaact tggaagtgtc		20
<210> 93 <211> 27 <212> DNA <213> Artificial Sequence		
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<400> 93 atagacagat gggggtgtcg ttttggc		27
<210> 94 <211> 23 <212> DNA <213> Artificial Sequence		
<220> <223> Description of Artificial Sequence: Synthetic primer		
<400> 94 cttgaccagg catcctagag tca		23
<210> 95 <211> 24 <212> DNA <213> Artificial Sequence		
<220> <223> Description of Artificial Sequence: Synthetic primer		
<400> 95 aggggccagt ggatagagtg atgg		24
<210> 96 <211> 100 <212> PRT <213> Mus sp.		
<400> 96 Glu Val Gln Leu Glu Glu ser Gly Gly Gly Leu Val Gln Pro Gly Gly 1 5 10 15		

Sequence Listing

Ala Leu Ser Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Thr Asp Tyr
20 25 30

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

Gly Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Ser Ala
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Gln Ser Ile
65 70 75 80

Leu Tyr Leu Gln Met Asn Ala Leu Arg Ala Glu Asp Ser Ala Thr Tyr
85 90 95

Tyr Cys Thr Arg
100

<210> 97
<211> 100
<212> PRT
<213> Homo sapiens

<400> 97
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp His
20 25 30

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

Gly Arg Thr Arg Asn Lys Ala Asn Ser Tyr Thr Thr Glu Tyr Ala Ala
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Ala Arg
100

<210> 98
<211> 100
<212> PRT
<213> Artificial sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

Sequence Listing

<400> 98

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

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

Gly Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Ser Ala
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Ala Arg
100

<210> 99

<211> 100

<212> PRT

<213> Mus sp.

<400> 99

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

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Thr
20 25 30

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

Pro Lys Leu Leu Ile Tyr Met Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

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

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

Ala His Val Pro
100

<210> 100

<211> 100

<212> PRT

<213> Homo sapiens

Sequence Listing

<400> 100

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

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asp Gly Lys Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Glu Val Ser Ser Arg Phe Ser Gly Val Pro
50 55 60

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

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Gly
85 90 95

Ile His Leu Pro
100

<210> 101

<211> 100

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 101

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

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Thr
20 25 30

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

Pro Gln Leu Leu Ile Tyr Met Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

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

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

Ala His Val Pro
100

<210> 102

Sequence Listing

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 102

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

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Thr
20 25 30

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

Pro Gln Leu Leu Ile Tyr Met Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

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

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

Ala His Val Pro Pro Leu Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile
100 105 110

Lys

<210> 103

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 103

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

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

Gly Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Ser Ala
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser

Sequence Listing

65

70

75

80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Ala Arg Tyr Ile Trp Phe Asp Tyr Trp Gly Gln Gly Thr Thr
100 105 110

Val Thr Val Ser Ser
115

<210> 104
<211> 12
<212> PRT
<213> Homo sapiens

<400> 104
Gly Gln Asn Asp Thr Ser Gln Thr Ser Ser Pro Ser
1 5 10

<210> 105
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 105
Ala Gln Asn Asp Thr Ser Gln Thr Ser Ser Pro Ser
1 5 10

<210> 106
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 106
Gly Ala Asn Asp Thr Ser Gln Thr Ser Ser Pro Ser
1 5 10

<210> 107
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 107
Gly Gln Ala Asp Thr Ser Gln Thr Ser Ser Pro Ser
1 5 10

<210> 108

Sequence Listing

<211> 12
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 108
 Gly Gln Asn Ala Thr Ser Gln Thr Ser Ser Pro Ser
 1 5 10

 <210> 109
 <211> 12
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 109
 Gly Gln Asn Asp Ala Ser Gln Thr Ser Ser Pro Ser
 1 5 10

 <210> 110
 <211> 12
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 110
 Gly Gln Asn Asp Thr Ala Gln Thr Ser Ser Pro Ser
 1 5 10

 <210> 111
 <211> 12
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 111
 Gly Gln Asn Asp Thr Ser Ala Thr Ser Ser Pro Ser
 1 5 10

 <210> 112
 <211> 12
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 112
 Gly Gln Asn Asp Thr Ser Gln Ala Ser Ser Pro Ser
 1 5 10

Sequence Listing

<210> 113
 <211> 12
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 113
 Gly Gln Asn Asp Thr Ser Gln Thr Ala Ser Pro Ser
 1 5 10

<210> 114
 <211> 12
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 114
 Gly Gln Asn Asp Thr Ser Gln Thr Ser Ala Pro Ser
 1 5 10

<210> 115
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<220>
 <221> MOD_RES
 <222> (1)..(1)
 <223> Lys or Arg

<220>
 <221> MOD_RES
 <222> (7)..(7)
 <223> Leu, Val or Ile

<220>
 <221> MOD_RES
 <222> (8)..(8)
 <223> Any amino acid

<220>
 <221> MOD_RES
 <222> (9)..(9)
 <223> Ser or Thr

<220>
 <221> MOD_RES
 <222> (10)..(10)
 <223> Asn or Asp

<220>
 <221> MOD_RES
 <222> (12)..(12)
 <223> Any amino acid

Sequence Listing

<220>
 <221> MOD_RES
 <222> (13)..(13)
 <223> Ser or Thr

<220>
 <221> MOD_RES
 <222> (16)..(16)
 <223> Any amino acid

<400> 115
 Xaa Ser Ser Gln Ser Leu Xaa Xaa Xaa Xaa Gly Xaa Xaa Tyr Leu Xaa
 1 5 10 15

<210> 116
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<220>
 <221> MOD_RES
 <222> (1)..(1)
 <223> Lys or Arg

<220>
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 <222> (7)..(7)
 <223> Leu, Val or Ile

<220>
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 <223> Ser or Thr

<220>
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 <222> (12)..(12)
 <223> Any amino acid

<220>
 <221> MOD_RES
 <222> (13)..(13)
 <223> Ser or Thr

<400> 116
 Xaa Ser Ser Gln Ser Leu Xaa His Xaa Asn Gly Xaa Xaa Tyr Leu His
 1 5 10 15

<210> 117
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<220>
 <221> MOD_RES

Sequence Listing

<222> (13)..(13)
 <223> Ser or Thr

<400> 117
 Arg Ser Ser Gln Ser Leu Val His Thr Asn Gly Asn Xaa Tyr Leu His
 1 5 10 15

<210> 118
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<220>
 <221> MOD_RES
 <222> (1)..(1)
 <223> Any amino acid

<220>
 <221> MOD_RES
 <222> (4)..(6)
 <223> Any amino acid

<400> 118
 Xaa Val Ser Xaa Xaa Xaa Ser
 1 5

<210> 119
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<220>
 <221> MOD_RES
 <222> (1)..(1)
 <223> Any amino acid

<220>
 <221> MOD_RES
 <222> (4)..(4)
 <223> Any amino acid

<220>
 <221> MOD_RES
 <222> (6)..(6)
 <223> Any amino acid

<400> 119
 Xaa Val Ser Xaa Arg Xaa Ser
 1 5

<210> 120
 <211> 7
 <212> PRT
 <213> Artificial Sequence

Sequence Listing

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<220>
 <221> MOD_RES
 <222> (4)..(4)
 <223> Any amino acid

<400> 120
 Met Val Ser Xaa Arg Phe Ser
 1 5

<210> 121
 <211> 8
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<220>
 <221> MOD_RES
 <222> (1)..(1)
 <223> Any amino acid

<220>
 <221> MOD_RES
 <222> (3)..(3)
 <223> Any amino acid

<220>
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 <222> (4)..(4)
 <223> Any amino acid

<220>
 <221> MOD_RES
 <222> (5)..(5)
 <223> His, Arg or Lys

<220>
 <221> MOD_RES
 <222> (6)..(6)
 <223> Phe, Leu, Val or Ile

<220>
 <221> MOD_RES
 <222> (7)..(8)
 <223> Any amino acid

<400> 121
 Xaa Gln Xaa Xaa Xaa Xaa Xaa Xaa
 1 5

<210> 122
 <211> 8
 <212> PRT
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<220>
 <223> Description of Artificial Sequence: Synthetic

Sequence Listing

peptide

<220>

<221> MOD_RES

<222> (4)..(4)

<223> Any amino acid

<220>

<221> MOD_RES

<222> (5)..(5)

<223> His, Arg or Lys

<220>

<221> MOD_RES

<222> (6)..(6)

<223> Phe, Leu, Val or Ile

<220>

<221> MOD_RES

<222> (8)..(8)

<223> Any amino acid

<400> 122

Ser Gln Ser Xaa Xaa Xaa Pro Xaa
1 5

<210> 123

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<220>

<221> MOD_RES

<222> (4)..(4)

<223> Any amino acid

<220>

<221> MOD_RES

<222> (8)..(8)

<223> Phe or Pro

<400> 123

Ser Gln Ser Xaa His Val Pro Xaa
1 5

<210> 124

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<220>

<221> MOD_RES

<222> (3)..(3)

<223> Any amino acid

Sequence Listing

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<220>
<221> MOD_RES
<222> (5)..(6)
<223> Any amino acid

<220>
<221> MOD_RES
<222> (8)..(8)
<223> Trp or Tyr

<220>
<221> MOD_RES
<222> (10)..(10)
<223> Any amino acid

<400> 124
Gly Phe Xaa Phe Xaa Xaa Tyr Xaa Met Xaa
1           5              10

<210> 125
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      peptide

<220>
<221> MOD_RES
<222> (5)..(6)
<223> Any amino acid

<220>
<221> MOD_RES
<222> (8)..(8)
<223> Trp or Tyr

<220>
<221> MOD_RES
<222> (10)..(10)
<223> Any amino acid

<400> 125
Gly Phe Thr Phe Xaa Xaa Tyr Xaa Met Xaa
1           5              10

<210> 126
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      peptide

<220>
<221> MOD_RES
<222> (8)..(8)
<223> Trp or Tyr

<400> 126
Gly Phe Thr Phe Thr Asp Tyr Xaa Met Ser
1           5              10

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Sequence Listing

<210> 127
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<220>
 <221> MOD_RES
 <222> (1)..(1)
 <223> Any amino acid

<220>
 <221> MOD_RES
 <222> (4)..(4)
 <223> Any amino acid

<220>
 <221> MOD_RES
 <222> (6)..(6)
 <223> Any amino acid

<220>
 <221> MOD_RES
 <222> (8)..(8)
 <223> Any amino acid

<220>
 <221> MOD_RES
 <222> (10)..(10)
 <223> Any amino acid

<220>
 <221> MOD_RES
 <222> (12)..(12)
 <223> Any amino acid

<220>
 <221> MOD_RES
 <222> (14)..(15)
 <223> Any amino acid

<400> 127
 Xaa Ile Arg Xaa Lys Xaa Asx Xaa Tyr Xaa Thr Xaa Tyr Xaa Xaa Ser
 1 5 10 15

Val Lys Gly

<210> 128
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<220>
 <221> MOD_RES

Sequence Listing

<222> (1)..(1)
 <223> Any amino acid

<220>
 <221> MOD_RES
 <222> (4)..(4)
 <223> Any amino acid

<220>
 <221> MOD_RES
 <222> (6)..(6)
 <223> Any amino acid

<220>
 <221> MOD_RES
 <222> (8)..(8)
 <223> Any amino acid

<220>
 <221> MOD_RES
 <222> (14)..(15)
 <223> Any amino acid

<400> 128
 Xaa Ile Arg Xaa Lys Xaa Asn Xaa Tyr Thr Thr Glu Tyr Xaa Xaa Ser
 1 5 10 15

Val Lys Gly

<210> 129
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<220>
 <221> MOD_RES
 <222> (14)..(15)
 <223> Any amino acid

<400> 129
 Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Xaa Xaa Ser
 1 5 10 15

Val Lys Gly

<210> 130
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<220>
 <221> MOD_RES

Sequence Listing

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<222> (2)..(4)
<223> Any amino acid

<220>
<221> MOD_RES
<222> (5)..(5)
<223> Tyr, Phe or Trp

<400> 130
Thr Xaa Xaa Xaa Xaa
1      5

<210> 131
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      peptide

<220>
<221> MOD_RES
<222> (4)..(4)
<223> Any amino acid

<220>
<221> MOD_RES
<222> (5)..(5)
<223> Tyr, Phe or Trp

<400> 131
Thr Arg Tyr Xaa Xaa Phe Asp Tyr
1      5

<210> 132
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      peptide

<220>
<221> MOD_RES
<222> (5)..(5)
<223> Phe or Trp

<400> 132
Thr Arg Tyr Ile Xaa Phe Asp Tyr
1      5

<210> 133
<211> 11
<212> PRT
<213> Homo sapiens

<400> 133
Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
1      5      10

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Sequence Listing

<210> 134
 <211> 10
 <212> PRT
 <213> Homo sapiens

<400> 134
 Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 1 5 10

<210> 135
 <211> 10
 <212> PRT
 <213> Homo sapiens

<400> 135
 Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys
 1 5 10

<210> 136
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic polypeptide

<400> 136
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Thr Asp Tyr
 20 25 30

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

Gly Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Asn Ala
 50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
 65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
 85 90 95

Tyr Cys Thr Arg Tyr Ile Phe Phe Asp Tyr Trp Gly Gln Gly Thr Thr
 100 105 110

Val Thr Val Ser Ser
 115

<210> 137
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>

Sequence Listing

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 137

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Thr Asp Tyr
20 25 30

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

Gly Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Asn Ala
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Ala Arg Tyr Ile Phe Phe Asp Tyr Trp Gly Gln Gly Thr Thr
100 105 110

Val Thr Val Ser Ser
115

<210> 138

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 138

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

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Thr
20 25 30

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

Pro Gln Leu Leu Ile Tyr Met Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

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

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

Sequence Listing

Thr His Val Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 139
<211> 114
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 139
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30

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

Gly Gln Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr His Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Thr Pro Val Asp Phe Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110

Ser Ser

<210> 140
<211> 114
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 140
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30

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

Sequence Listing

Gly Gln Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr His Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
65 70 75 80

Val Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Thr Pro Val Asp Phe Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110

Ser Ser

<210> 141

<211> 114

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 141

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30

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

Ala Gln Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr His Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Thr Pro Val Asp Phe Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110

Ser Ser

<210> 142

<211> 114

<212> PRT

<213> Artificial Sequence

Sequence Listing

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 142

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30

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

Ala Gln Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr His Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
65 70 75 80

Val Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Thr Pro Val Asp Phe Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110

Ser Ser

<210> 143

<211> 114

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 143

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

Ser Met Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30

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

Ala Gln Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr His Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
65 70 75 80

Sequence Listing

Val Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Thr Pro Val Asp Phe Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110

Ser Ser

<210> 144

<211> 114

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 144

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

Ser Met Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
20 25 30

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

Ala Gln Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr His Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
65 70 75 80

Val Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Thr Pro Val Asp Phe Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110

Ser Ser

<210> 145

<211> 111

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 145

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

Sequence Listing

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

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

Pro Gln Leu Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

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

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

Thr His Leu His Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys
100 105 110

<210> 146

<211> 111

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 146

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

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

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

Pro Gln Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

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

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

Thr His Leu His Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys
100 105 110

<210> 147

<211> 111

<212> PRT

<213> Artificial Sequence

<220>

Sequence Listing

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 147

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

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

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

Pro Gln Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

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

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

Thr His Leu His Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys
100 105 110

<210> 148

<211> 111

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 148

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

Gln Pro Val Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

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

Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

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

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

Thr His Leu His Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys
100 105 110

Sequence Listing

<210> 149
 <211> 114
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic polypeptide

<400> 149
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Pro Phe Ser Asn Tyr
 20 25 30
 Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Gly Gln Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr His Tyr Ala Glu
 50 55 60
 Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
 65 70 75 80
 Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
 85 90 95
 Tyr Cys Thr Pro Ile Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
 100 105 110
 Ser Ser

<210> 150
 <211> 114
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic polypeptide

<400> 150
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Pro Phe Ser Asn Tyr
 20 25 30
 Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Gly Gln Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr His Tyr Ala Glu
 50 55 60

Sequence Listing

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
65 70 75 80

Val Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Thr Pro Ile Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110

Ser Ser

<210> 151

<211> 114

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 151

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Pro Phe Ser Asn Tyr
20 25 30

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

Ala Gln Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr His Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Thr Pro Ile Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110

Ser Ser

<210> 152

<211> 114

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

Sequence Listing

<400> 152
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Pro Phe Ser Asn Tyr
 20 25 30
 Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Gln Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr His Tyr Ala Glu
 50 55 60
 Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
 65 70 75 80
 Val Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
 85 90 95
 Tyr Cys Thr Pro Ile Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
 100 105 110
 Ser Ser

<210> 153
 <211> 111
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic polypeptide

<400> 153
 Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
 1 5 10 15
 Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
 20 25 30
 Asn Gly Lys Thr Tyr Leu Asn Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45
 Pro Gln Leu Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
 50 55 60
 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80
 Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Val Gln Gly
 85 90 95

Sequence Listing

Ser His Phe His Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 100 105 110

<210> 154
 <211> 111
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic polypeptide

<400> 154
 Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
 1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
 20 25 30

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

Pro Gln Leu Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
 50 55 60

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

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

Ser His Phe His Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 100 105 110

<210> 155
 <211> 111
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic polypeptide

<400> 155
 Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
 1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
 20 25 30

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

Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
 50 55 60

Sequence Listing

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

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

Ser His Phe His Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 156

<211> 111

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 156

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

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

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

Pro Gln Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

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

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

Ser His Phe His Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 157

<211> 111

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 157

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

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

Sequence Listing

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

Pro Gln Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

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

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

Ser His Phe His Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 158

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 158

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ala
20 25 30

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

Ser Glu Ile Arg Asn Lys Ala Lys Asn His Ala Thr Tyr Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
65 70 75 80

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

Tyr Cys Thr Thr Leu Asp Ser Trp Gly Gln Thr Thr Val Thr Val Ser
100 105 110

Ser

<210> 159

<211> 114

<212> PRT

<213> Artificial Sequence

<220>

Sequence Listing

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 159

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ala
20 25 30

Trp Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Thr
35 40 45

Ala Glu Ile Arg Asn Lys Ala Lys Asn His Ala Thr Tyr Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ser Thr
65 70 75 80

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

Tyr Cys Thr Thr Leu Asp Ser Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110

Ser Ser

<210> 160

<211> 114

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 160

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ala
20 25 30

Trp Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Thr
35 40 45

Ala Glu Ile Arg Asn Lys Ala Lys Asn His Ala Thr Tyr Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
65 70 75 80

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

Sequence Listing

Tyr Cys Thr Thr Leu Asp Ser Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110

Ser Ser

<210> 161
<211> 114
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 161
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ala
20 25 30

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

Ser Glu Ile Arg Asn Lys Ala Lys Asn His Ala Thr Tyr Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
65 70 75 80

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

Tyr Cys Thr Thr Leu Asp Ser Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110

Ser Ser

<210> 162
<211> 114
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 162
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ala
20 25 30

Sequence Listing

Trp Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Thr
 35 40 45

Ser Glu Ile Arg Asn Lys Ala Lys Asn His Ala Thr Tyr Tyr Ala Glu
 50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
 65 70 75 80

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

Tyr Cys Thr Thr Leu Asp Ser Trp Gly Gln Gly Thr Thr Val Thr Val
 100 105 110

Ser Ser

<210> 163

<211> 114

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 163

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ala
 20 25 30

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

Ala Glu Ile Arg Asn Lys Ala Lys Asn His Ala Thr Tyr Tyr Ala Glu
 50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
 65 70 75 80

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

Tyr Cys Thr Thr Leu Asp Ser Trp Gly Gln Gly Thr Thr Val Thr Val
 100 105 110

Ser Ser

Sequence Listing

<210> 164

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 164

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

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30

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

Pro Gln Leu Leu Ile Tyr Leu Val Ser Ala Leu Asp Ser Gly Val Pro
50 55 60

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

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Trp Gln Gly
85 90 95

Thr His Phe Pro Trp Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 165

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 165

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

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30

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

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

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

Sequence Listing

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Trp Gln Gly
85 90 95

Thr His Phe Pro Trp Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 166

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 166

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

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30

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

Pro Gln Arg Leu Ile Tyr Leu Val Ser Ala Leu Asp Ser Gly Val Pro
50 55 60

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

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Trp Gln Gly
85 90 95

Thr His Phe Pro Trp Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 167

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 167

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

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30

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

Sequence Listing

Pro Gln Arg Leu Ile Tyr Leu Val Ser Ala Leu Asp Ser Gly Val Pro
50 55 60

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

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Trp Gln Gly
85 90 95

Thr His Phe Pro Trp Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 168

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 168

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

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30

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

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

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

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Trp Gln Gly
85 90 95

Thr His Phe Pro Trp Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 169

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 169

Ala Gln Asn Asp Thr Ser Gln Thr Ser Ser Pro Ser Ala Asp Cys
1 5 10 15

Sequence Listing

<210> 170
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 170
 Gly Ala Asn Asp Thr Ser Gln Thr Ser Ser Pro Ser Ala Asp Cys
 1 5 10 15

 <210> 171
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 171
 Gly Gln Ala Asp Thr Ser Gln Thr Ser Ser Pro Ser Ala Asp Cys
 1 5 10 15

 <210> 172
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 172
 Gly Gln Asn Ala Thr Ser Gln Thr Ser Ser Pro Ser Ala Asp Cys
 1 5 10 15

 <210> 173
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 173
 Gly Gln Asn Asp Ala Ser Gln Thr Ser Ser Pro Ser Ala Asp Cys
 1 5 10 15

 <210> 174
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 174
 Gly Gln Asn Asp Thr Ala Gln Thr Ser Ser Pro Ser Ala Asp Cys
 1 5 10 15

Sequence Listing

<210> 175
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 175
 Gly Gln Asn Asp Thr Ser Ala Thr Ser Ser Pro Ser Ala Asp Cys
 1 5 10 15

<210> 176
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 176
 Gly Gln Asn Asp Thr Ser Gln Ala Ser Ser Pro Ser Ala Asp Cys
 1 5 10 15

<210> 177
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 177
 Gly Gln Asn Asp Thr Ser Gln Thr Ala Ser Pro Ser Ala Asp Cys
 1 5 10 15

<210> 178
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 178
 Gly Gln Asn Asp Thr Ser Gln Thr Ser Ala Pro Ser Ala Asp Cys
 1 5 10 15

<210> 179
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 179

Sequence Listing

Gly Gln Asn Asp Thr Ser Gln Thr Ser Ser Ala Ser Ala Asp Cys
1 5 10 15

<210> 180
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 180
Gly Gln Asn Asp Thr Ser Gln Thr Ser Ser Pro Ala Ala Asp Cys
1 5 10 15

<210> 181
<211> 15
<212> PRT
<213> Homo sapiens

<400> 181
Gly Gln Asn Asp Thr Ser Gln Thr Ser Ser Ala Ser Ala Asp Cys
1 5 10 15

<210> 182
<211> 15
<212> PRT
<213> Homo sapiens

<400> 182
Gly Gln Asn Asp Thr Ser Gln Thr Ser Ser Pro Ala Ala Asp Cys
1 5 10 15

<210> 183
<211> 19
<212> PRT
<213> Unknown

<220>
<223> Description of Unknown: Tetanus toxoid peptide

<400> 183
Asp Thr Ile Met Met Glu Pro Pro Tyr Cys Lys Gly Leu Asp Ile Tyr
1 5 10 15

Tyr Lys Ala

<210> 184
<211> 21
<212> PRT
<213> Unknown

<220>
<223> Description of Unknown: Tetanus toxoid peptide

<400> 184
Ser Ala Met Leu Thr Asn Leu Ile Ile Phe Gly Pro Gly Pro Val Leu
1 5 10 15

Sequence Listing

Asn Lys Asn Glu Val
20

<210> 185
<211> 20
<212> PRT
<213> Unknown

<220>
<223> Description of Unknown: Tetanus toxoid peptide

<400> 185
Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys Val Ser Ala
1 5 10 15

Ser His Leu Glu
20

<210> 186
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 186
Asp Ile Glu Lys Lys Ile Ala Lys Met Glu Lys Ala Ser Ser Val Phe
1 5 10 15

Asn Val Val Asn Ser
20

<210> 187
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 187
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

<210> 188
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 188
Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr
1 5 10 15

<210> 189

Sequence Listing

<211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 189
 Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala
 1 5 10 15

 <210> 190
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 190
 Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Ile Asp
 1 5 10 15

 <210> 191
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 191
 Ile Thr Cys Lys Ala Ser Gln Asn Ile Asp Lys Tyr Leu Asn Trp
 1 5 10 15

 <210> 192
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 192
 Ser Gln Asn Ile Asp Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro
 1 5 10 15

 <210> 193
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 193
 Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
 1 5 10 15

Sequence Listing

<210> 194
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 194
 Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Asn
 1 5 10 15

<210> 195
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 195
 Gly Lys Ala Pro Lys Leu Leu Ile Tyr Asn Thr Asn Asn Leu Gln
 1 5 10 15

<210> 196
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 196
 Leu Leu Ile Tyr Asn Thr Asn Asn Leu Gln Thr Gly Val Pro Ser
 1 5 10 15

<210> 197
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 197
 Thr Asn Asn Leu Gln Thr Gly Val Pro Ser Arg Phe Ser Gly Ser
 1 5 10 15

<210> 198
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 198
 Thr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
 Page 70

		Sequence Listing	
1	5	10	15
<210>	199		
<211>	15		
<212>	PRT		
<213>	Artificial Sequence		
<220>			
<223>	Description of Artificial Sequence: Synthetic peptide		
<400>	199		
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile			
1	5	10	15
<210>	200		
<211>	15		
<212>	PRT		
<213>	Artificial Sequence		
<220>			
<223>	Description of Artificial Sequence: Synthetic peptide		
<400>	200		
Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro			
1	5	10	15
<210>	201		
<211>	15		
<212>	PRT		
<213>	Artificial Sequence		
<220>			
<223>	Description of Artificial Sequence: Synthetic peptide		
<400>	201		
Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr			
1	5	10	15
<210>	202		
<211>	15		
<212>	PRT		
<213>	Artificial Sequence		
<220>			
<223>	Description of Artificial Sequence: Synthetic peptide		
<400>	202		
Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln			
1	5	10	15
<210>	203		
<211>	15		
<212>	PRT		
<213>	Artificial Sequence		
<220>			
<223>	Description of Artificial Sequence: Synthetic peptide		

Sequence Listing

<400> 203
 Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln His Ile Ser Arg Pro
 1 5 10 15

<210> 204
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 204
 Tyr Tyr Cys Leu Gln His Ile Ser Arg Pro Arg Thr Phe Gly Gln
 1 5 10 15

<210> 205
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 205
 His Ile Ser Arg Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu
 1 5 10 15

<210> 206
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 206
 Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val
 1 5 10 15

<210> 207
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 207
 Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser
 1 5 10 15

<210> 208
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic
 Page 72

Sequence Listing

peptide

<400> 208
 Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln Thr Leu Ser Leu
 1 5 10 15

<210> 209
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 209
 Leu Val Arg Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser
 1 5 10 15

<210> 210
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 210
 Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Thr
 1 5 10 15

<210> 211
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 211
 Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Asp Phe Tyr Met Asn
 1 5 10 15

<210> 212
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 212
 Gly Phe Thr Phe Thr Asp Phe Tyr Met Asn Trp Val Arg Gln Pro
 1 5 10 15

<210> 213
 <211> 15
 <212> PRT
 <213> Artificial Sequence

Sequence Listing

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 213

Asp	Phe	Tyr	Met	Asn	Trp	Val	Arg	Gln	Pro	Pro	Gly	Arg	Gly	Leu
1				5					10					15

<210> 214

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 214

Trp	Val	Arg	Gln	Pro	Pro	Gly	Arg	Gly	Leu	Glu	Trp	Ile	Gly	Phe
1				5					10					15

<210> 215

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 215

Pro	Gly	Arg	Gly	Leu	Glu	Trp	Ile	Gly	Phe	Ile	Arg	Asp	Lys	Ala
1				5					10					15

<210> 216

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 216

Glu	Trp	Ile	Gly	Phe	Ile	Arg	Asp	Lys	Ala	Lys	Gly	Tyr	Thr	Thr
1				5					10					15

<210> 217

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 217

Ile	Arg	Asp	Lys	Ala	Lys	Gly	Tyr	Thr	Thr	Glu	Tyr	Asn	Pro	Ser
1				5					10					15

<210> 218

<211> 15

<212> PRT

Sequence Listing

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 218

Lys	Gly	Tyr	Thr	Thr	Glu	Tyr	Asn	Pro	Ser	Val	Lys	Gly	Arg	Val
1				5					10					15

<210> 219

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 219

Glu	Tyr	Asn	Pro	Ser	Val	Lys	Gly	Arg	Val	Thr	Met	Leu	Val	Asp
1				5					10					15

<210> 220

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 220

Val	Lys	Gly	Arg	Val	Thr	Met	Leu	Val	Asp	Thr	Ser	Lys	Asn	Gln
1				5					10					15

<210> 221

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 221

Thr	Met	Leu	Val	Asp	Thr	Ser	Lys	Asn	Gln	Phe	Ser	Leu	Arg	Leu
1				5					10					15

<210> 222

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 222

Thr	Ser	Lys	Asn	Gln	Phe	Ser	Leu	Arg	Leu	Ser	Ser	Val	Thr	Ala
1				5					10					15

<210> 223

Sequence Listing

<211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 223
 Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
 1 5 10 15

 <210> 224
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 224
 Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg
 1 5 10 15

 <210> 225
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 225
 Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg Glu Gly His Thr Ala
 1 5 10 15

 <210> 226
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 226
 Tyr Tyr Cys Ala Arg Glu Gly His Thr Ala Ala Pro Phe Asp Tyr
 1 5 10 15

 <210> 227
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 227
 Glu Gly His Thr Ala Ala Pro Phe Asp Tyr Trp Gly Gln Gly Ser
 1 5 10 15

Sequence Listing

<210> 228
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 228
 Ala Pro Phe Asp Tyr Trp Gly Gln Gly Ser Leu Val Thr Val Ser
 1 5 10 15

<210> 229
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 229
 Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys
 1 5 10 15

<210> 230
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 230
 Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro
 1 5 10 15

<210> 231
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 231
 Gln Thr Pro Leu Ser Leu Val Thr Pro Gly Gln Pro Ala Ser
 1 5 10

<210> 232
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 232
 Leu Ser Val Thr Pro Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser
 Page 77

1	5	Sequence Listing 10	15
<210>	233		
<211>	15		
<212>	PRT		
<213>	Artificial Sequence		
<220>			
<223>	Description of Artificial Sequence: Synthetic peptide		
<400>	233		
Gly	Gln	Pro	Ala
1	5	Ser	Ile
		Ser	Cys
		Lys	Ser
		Ser	Ser
		Gln	Ser
		Leu	Leu
		10	15
<210>	234		
<211>	15		
<212>	PRT		
<213>	Artificial Sequence		
<220>			
<223>	Description of Artificial Sequence: Synthetic peptide		
<400>	234		
Ile	Ser	Cys	Lys
1	5	Ser	Ser
		Gln	Ser
		Leu	Leu
		Tyr	Ser
		Asn	Gly
		Lys	
		10	15
<210>	235		
<211>	15		
<212>	PRT		
<213>	Artificial Sequence		
<220>			
<223>	Description of Artificial Sequence: Synthetic peptide		
<400>	235		
Ser	Gln	Ser	Leu
1	5	Leu	Tyr
		Ser	Asn
		Gly	Lys
		Thr	Tyr
		Leu	Asn
		Trp	
		10	15
<210>	236		
<211>	15		
<212>	PRT		
<213>	Artificial Sequence		
<220>			
<223>	Description of Artificial Sequence: Synthetic peptide		
<400>	236		
Tyr	Ser	Asn	Gly
1	5	Lys	Thr
		Tyr	Leu
		Asn	Trp
		Val	Leu
		Gln	Lys
		Pro	
		10	15
<210>	237		
<211>	15		
<212>	PRT		
<213>	Artificial Sequence		
<220>			
<223>	Description of Artificial Sequence: Synthetic peptide		

Sequence Listing

<400> 237

Thr Tyr Leu Asn Trp Val Leu Gln Lys Pro Gly Gln Ser Pro Gln
1 5 10 15

<210> 238

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 238

Val Leu Gln Lys Pro Gly Gln Ser Pro Gln Arg Leu Ile Tyr Leu
1 5 10 15

<210> 239

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 239

Gly Gln Ser Pro Gln Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp
1 5 10 15

<210> 240

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 240

Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro Asp
1 5 10 15

<210> 241

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 241

Val Ser Lys Leu Asp Ser Gly Val Pro Asp Arg Phe Ser Gly Ser
1 5 10 15

<210> 242

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

Sequence Listing

peptide

<400> 242

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
1 5 10 15

<210> 243

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 243

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
1 5 10 15

<210> 244

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 244

Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala
1 5 10 15

<210> 245

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 245

Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val
1 5 10 15

<210> 246

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 246

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Val Gln
1 5 10 15

<210> 247

<211> 15

<212> PRT

<213> Artificial Sequence

Sequence Listing

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 247

Glu Asp Val Gly Val Tyr Tyr Cys Val Gln Gly Ser His Phe His
1 5 10 15

<210> 248

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 248

Tyr Tyr Cys Val Gln Gly Ser His Phe His Thr Phe Gly Gln Gly
1 5 10 15

<210> 249

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 249

Gly Ser His Phe His Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
1 5 10 15

<210> 250

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 250

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala
1 5 10 15

<210> 251

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 251

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

<210> 252

<211> 15

<212> PRT

Sequence Listing

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 252

Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu
1 5 10 15

<210> 253

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 253

Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser
1 5 10 15

<210> 254

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 254

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Pro Phe Ser
1 5 10 15

<210> 255

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 255

Ser Cys Ala Ala Ser Gly Phe Pro Phe Ser Asn Tyr Trp Met Asn
1 5 10 15

<210> 256

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 256

Gly Phe Pro Phe Ser Asn Tyr Trp Met Asn Trp Val Arg Gln Ala
1 5 10 15

<210> 257

Sequence Listing

<211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 257
 Asn Tyr Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
 1 5 10 15

 <210> 258
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 258
 Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Gly Gln
 1 5 10 15

 <210> 259
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 259
 Pro Gly Lys Gly Leu Glu Trp Val Gly Gln Ile Arg Leu Lys Ser
 1 5 10 15

 <210> 260
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 260
 Glu Trp Val Gly Gln Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr
 1 5 10 15

 <210> 261
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic peptide

 <400> 261
 Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr His Tyr Ala Glu Ser
 1 5 10 15

Sequence Listing

<210> 262
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 262
 Asn Asn Tyr Ala Thr His Tyr Ala Glu Ser Val Lys Gly Arg Phe
 1 5 10 15

<210> 263
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 263
 His Tyr Ala Glu Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp
 1 5 10 15

<210> 264
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 264
 Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
 1 5 10 15

<210> 265
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 265
 Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser Leu Tyr Leu Gln Met
 1 5 10 15

<210> 266
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 266
 Asp Ser Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr

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<210>	267		
<211>	15		
<212>	PRT		
<213>	Artificial Sequence		
<220>			
<223>	Description of Artificial Sequence: Synthetic peptide		
<400>	267		
Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val			
1 5 10 15			
<210>	268		
<211>	15		
<212>	PRT		
<213>	Artificial Sequence		
<220>			
<223>	Description of Artificial Sequence: Synthetic peptide		
<400>	268		
Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr Tyr Cys Thr Pro			
1 5 10 15			
<210>	269		
<211>	15		
<212>	PRT		
<213>	Artificial Sequence		
<220>			
<223>	Description of Artificial Sequence: Synthetic peptide		
<400>	269		
Glu Asp Thr Ala Val Tyr Tyr Cys Thr Pro Ile Asp Tyr Trp Gly			
1 5 10 15			
<210>	270		
<211>	15		
<212>	PRT		
<213>	Artificial Sequence		
<220>			
<223>	Description of Artificial Sequence: Synthetic peptide		
<400>	270		
Tyr Tyr Cys Thr Pro Ile Asp Tyr Trp Gly Gln Gly Thr Thr Val			
1 5 10 15			
<210>	271		
<211>	15		
<212>	PRT		
<213>	Artificial Sequence		
<220>			
<223>	Description of Artificial Sequence: Synthetic peptide		

Sequence Listing

<400> 271

Ile Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala
1 5 10 15

<210> 272

<211> 464

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 272

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

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

Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
35 40 45

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

Leu Glu Trp Val Gly Gln Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr
65 70 75 80

His Tyr Ala Glu Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp
85 90 95

Ser Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp
100 105 110

Thr Ala Val Tyr Tyr Cys Thr Pro Val Asp Phe Trp Gly Gln Gly Thr
115 120 125

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

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

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
165 170 175

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

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

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser

Sequence Listing

210

215

220

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

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
245 250 255

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

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

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

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

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
325 330 335

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

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

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

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

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
405 410 415

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

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
435 440 445

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 273

<211> 238

<212> PRT

<213> Artificial Sequence

Sequence Listing

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 273

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Trp Leu Pro
1 5 10 15

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

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

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

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

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

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
100 105 110

Cys Val Gln Gly Thr His Leu His Thr Phe Gly Gln Gly Thr Arg Leu
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> 274

<211> 467

<212> PRT

Sequence Listing

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 274

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

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

Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
35 40 45

Phe Thr Asp Tyr Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly
50 55 60

Leu Glu Trp Val Gly Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr
65 70 75 80

Glu Tyr Asn Ala Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp
85 90 95

Ser Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp
100 105 110

Thr Ala Val Tyr Tyr Cys Thr Arg Tyr Ile Phe Phe 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

Sequence Listing

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> 275

<211> 239

<212> PRT

<213> Artificial sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

Sequence Listing

<400> 275

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

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

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

Leu Val His Thr Asn Gly Asn Ser Tyr Leu His Trp Tyr Leu Gln Lys
50 55 60

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Met Val Ser Asn Arg Phe
65 70 75 80

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

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
100 105 110

Cys Ser Gln Ser Thr His Val Pro Phe Thr Phe Gly Gln Gly Thr Lys
115 120 125

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

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

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

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

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

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

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

<210> 276

<211> 464

<212> PRT

<213> Artificial sequence

<220>

<223> Description of Artificial sequence: Synthetic

polypeptide

Sequence Listing

<400> 276

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

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

Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
35 40 45

Phe Ser Asp Ala Trp Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly
50 55 60

Leu Glu Leu Val Ser Glu Ile Arg Asn Lys Ala Lys Asn His Ala Thr
65 70 75 80

Tyr Tyr Ala Glu Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
85 90 95

Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
100 105 110

Thr Ala Val Tyr Tyr Cys Thr Thr Leu Asp Ser Trp Gly Gln Gly Thr
115 120 125

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

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

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
165 170 175

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

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

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

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

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
245 250 255

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg

Sequence Listing

260 265 270

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

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

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

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
325 330 335

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

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

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

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

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
405 410 415

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

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
435 440 445

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 277
<211> 464
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 277
Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

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

Sequence Listing

Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
35 40 45

Phe Ser Asp Ala Trp Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly
50 55 60

Leu Glu Leu Val Ala Glu Ile Arg Asn Lys Ala Lys Asn His Ala Thr
65 70 75 80

Tyr Tyr Ala Glu Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
85 90 95

Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
100 105 110

Thr Ala Val Tyr Tyr Cys Thr Thr Leu Asp Ser Trp Gly Gln Gly Thr
115 120 125

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

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

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
165 170 175

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

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

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

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

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
245 250 255

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

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

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

Sequence Listing

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

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
325 330 335

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

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

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

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

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
405 410 415

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

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
435 440 445

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 278

<211> 239

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 278

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

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

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

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

Pro Gly Gln Ser Pro Gln Arg Leu Ile Tyr Leu Val Ser Ala Leu Asp
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Sequence Listing															
65				70				75				80			
Ser	Gly	Val	Pro	Asp 85	Arg	Phe	Ser	Gly	Ser 90	Gly	Ser	Gly	Thr	Asp 95	Phe
Thr	Leu	Lys	Ile 100	Ser	Arg	Val	Glu	Ala 105	Glu	Asp	Val	Gly	Val 110	Tyr	Tyr
Cys	Trp	Gln 115	Gly	Thr	His	Phe	Pro 120	Trp	Thr	Phe	Gly	Gln 125	Gly	Thr	Lys
Leu	Glu 130	Ile	Lys	Arg	Thr	Val 135	Ala	Ala	Pro	Ser	Val 140	Phe	Ile	Phe	Pro
Pro 145	Ser	Asp	Glu	Gln	Leu 150	Lys	Ser	Gly	Thr	Ala 155	Ser	Val	Val	Cys	Leu 160
Leu	Asn	Asn	Phe	Tyr 165	Pro	Arg	Glu	Ala	Lys 170	Val	Gln	Trp	Lys	Val 175	Asp
Asn	Ala	Leu	Gln 180	Ser	Gly	Asn	Ser	Gln 185	Glu	Ser	Val	Thr	Glu 190	Gln	Asp
Ser	Lys	Asp 195	Ser	Thr	Tyr	Ser	Leu 200	Ser	Ser	Thr	Leu 205	Thr	Leu	Ser	Lys
Ala	Asp 210	Tyr	Glu	Lys	His	Lys 215	Val	Tyr	Ala	Cys	Glu 220	Val	Thr	His	Gln
Gly 225	Leu	Ser	Ser	Pro	Val 230	Thr	Lys	Ser	Phe	Asn 235	Arg	Gly	Glu	Cys	
<210> 279															
<211> 464															
<212> PRT															
<213> Artificial Sequence															
<220>															
<223> Description of Artificial Sequence: Synthetic polypeptide															
<400> 279															
Met 1	Glu	Ala	Pro	Ala 5	Gln	Leu	Leu	Phe	Leu 10	Leu	Leu	Leu	Trp	Leu 15	Pro
Asp	Thr	Thr	Gly 20	Glu	Val	Gln	Leu	Val 25	Glu	Ser	Gly	Gly	Gly 30	Leu	Val
Gln	Pro	Gly 35	Gly	Ser	Leu	Arg	Leu 40	Ser	Cys	Ala	Ala	Ser 45	Gly	Phe	Pro
Phe	Ser 50	Asn	Tyr	Trp	Met	Asn 55	Trp	Val	Arg	Gln 60	Ala	Pro	Gly	Lys	Gly

Sequence Listing

Leu Glu Trp Val Gly Gln Ile Arg Leu Lys Ser Asn Asn Tyr Ala Thr
 65 70 75 80
 His Tyr Ala Glu Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp
 85 90 95
 Ser Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp
 100 105 110
 Thr Ala Val Tyr Tyr Cys Thr Pro Ile Asp Tyr Trp Gly Gln Gly Thr
 115 120 125
 Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
 130 135 140
 Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
 145 150 155 160
 Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
 165 170 175
 Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
 180 185 190
 Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
 195 200 205
 Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
 210 215 220
 Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
 225 230 235 240
 His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
 245 250 255
 Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
 260 265 270
 Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
 275 280 285
 Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
 290 295 300
 Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
 305 310 315 320
 Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
 325 330 335

Sequence Listing

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

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

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

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

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
405 410 415

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

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
435 440 445

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 280
<211> 238
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 280
Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

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

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

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

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

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

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
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Sequence Listing

100

105

110

Cys Val Gln Gly Ser His Phe His Thr Phe Gly Gln Gly Thr Lys Leu
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> 281

<211> 467

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 281

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

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

Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
35 40 45

Phe Ser Asp Tyr Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly
50 55 60

Leu Glu Trp Val Gly Phe Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr
65 70 75 80

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

Sequence Listing

Ser Lys Asn Ser₁₀₀ Leu Tyr Leu Gln Met₁₀₅ Asn Ser Leu Lys Thr₁₁₀ Glu Asp

Thr Ala Val₁₁₅ Tyr Tyr Cys Ala Arg₁₂₀ Tyr Ile Trp Phe Asp₁₂₅ Tyr Trp Gly

Gln Gly₁₃₀ Thr Thr Val Thr Val₁₃₅ Ser Ser Ala Ser Thr₁₄₀ Lys Gly Pro Ser

Val₁₄₅ Phe Pro Leu Ala Pro₁₅₀ Ser Ser Lys Ser Thr₁₅₅ Ser Gly Gly Thr Ala₁₆₀

Ala Leu Gly Cys Leu₁₆₅ Val Lys Asp Tyr Phe₁₇₀ Pro Glu Pro Val Thr₁₇₅ Val

Ser Trp Asn Ser₁₈₀ Gly Ala Leu Thr Ser₁₈₅ Gly Val His Thr Phe₁₉₀ Pro Ala

Val Leu Gln₁₉₅ Ser Ser Gly Leu Tyr₂₀₀ Ser Leu Ser Ser Val₂₀₅ Val Thr Val

Pro Ser₂₁₀ Ser Ser Leu Gly Thr₂₁₅ Gln Thr Tyr Ile Cys₂₂₀ Asn Val Asn His

Lys₂₂₅ Pro Ser Asn Thr Lys₂₃₀ Val Asp Lys Lys Val₂₃₅ Glu Pro Lys Ser Cys₂₄₀

Asp Lys Thr His Thr₂₄₅ Cys Pro Pro Cys Pro₂₅₀ Ala Pro Glu Leu Leu₂₅₅ Gly

Gly Pro Ser Val₂₆₀ Phe Leu Phe Pro Pro₂₆₅ Lys Pro Lys Asp Thr₂₇₀ Leu Met

Ile Ser Arg₂₇₅ Thr Pro Glu Val Thr₂₈₀ Cys Val Val Val Asp₂₈₅ Val Ser His

Glu Asp₂₉₀ Pro Glu Val Lys Phe₂₉₅ Asn Trp Tyr Val Asp₃₀₀ Gly Val Glu Val

His₃₀₅ Asn Ala Lys Thr Lys₃₁₀ Pro Arg Glu Glu Gln₃₁₅ Tyr Asn Ser Thr Tyr₃₂₀

Arg Val Val Ser Val₃₂₅ Leu Thr Val Leu His₃₃₀ Gln Asp Trp Leu Asn₃₃₅ Gly

Lys Glu Tyr Lys₃₄₀ Cys Lys Val Ser Asn₃₄₅ Lys Ala Leu Pro Ala₃₅₀ Pro Ile

Glu Lys Thr₃₅₅ Ile Ser Lys Ala Lys₃₆₀ Gly Gln Pro Arg Glu₃₆₅ Pro Gln Val

Sequence Listing

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> 282

<211> 240

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 282

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

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

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

Leu Val His Thr Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys
50 55 60

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Met Val Ser Asn Arg Phe
65 70 75 80

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

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
100 105 110

Cys Ser Gln Ser Ala His Val Pro Pro Leu Thr Phe Gly Gln Gly Thr

Sequence Listing

115

120

125

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

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

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

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

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

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

Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235 240

<210> 283

<211> 1395

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 283

atggaagccc cagcgagct tctcttctc ctgctactct ggctccctga taccaccgga	60
gaggtacagc tgggtggagtc gggaggaggc ttggtacagc ctgggggttc tctgagactc	120
tcctgtgcag cttctggatt cactttcaat acctactgga tgaactgggt ccgccaggct	180
ccaggggaagg gacttgagtg ggtgggtcaa attagattga aatctaataa ttatgcaaca	240
cattatgcgg agtctgtgaa agggcggttc accatctcca gagatgattc caaaaacagc	300
ctctatcttc aaatgaattc cctgaaaact gaagacactg ccgtttatta ctgtacccca	360
gttgactttt ggggccaagg caccactgtc acagtctcct cagcctccac caagggccca	420
tcggtcttcc ccctggcacc ctctccaag agcacctctg ggggtacagc ggccctgggc	480
tgcttggtca aggactactt ccccgaaccg gtgacggtgt cgtggaactc aggcgcctg	540
accagcggcg tgcacacctt cccggctgtc ctacagtcct caggactcta ctccctcagc	600
agcgtggtga ccgtgccctc cagcagcttg ggcaccaga cctacatctg caacgtgaat	660
cacaagcca gcaacaccaa ggtggacaag aaagttgagc ccaaattctg tgacaaaact	720
cacacatgcc caccgtgccc agcacctgaa ctctggggg gaccgtcagt cttctcttc	780
ccccaaaac ccaaggacac cctcatgatc tcccggaacc ctgaggtcac atgcgtggtg	840

Sequence Listing

gtggacgtga gccacgaaga ccctgaggtc aagttcaact ggtacgtgga cggcgtggag	900
gtgcataatg ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtggtc	960
agcgtcctca ccgtcctgca ccaggactgg ctgaatggca aggagtacaa gtgcaaggtc	1020
tccaacaaag ccctcccagc ccccatcgag aaaaccatct ccaaagccaa agggcagccc	1080
cgagaaccac aggtgtacac cctgccccca tcccgggatg agctgaccaa gaaccaggtc	1140
agcctgacat gcctgggtcaa aggcttctat cccagcgaca tcgccgtgga gtgggagagc	1200
aatgggcagc cggagaacaa ctacaagacc acgcctcccg tgctggactc cgacggctcc	1260
ttcttcctct acagcaagct caccgtggac aagtccaggt ggcagcaggg gaacgtcttc	1320
tcatgctccg tgatgcatga ggctctgcac aaccactaca cgagaagag cctctccctg	1380
tctccgggta aatga	1395

<210> 284

<211> 717

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 284

atggaagccc cagcgcagct tctcttcctc ctgctactct ggctccctga taccaccgga	60
gacattgtga tgaccagac tccactcagt ttgtcagtta cccctggaca accagcctca	120
atctcttgca agtcaagtca gagcctctta tatagtaatg gaaaaaccta tttgaactgg	180
ttattacaga agccaggcca gtctccacag cgcctaactct atctgggtgtc taaattggac	240
tctggagtcc ctgacagggt cagtggcagt ggatcaggaa cagattttac actgaaaatc	300
agcagagtgg aggctgagga tgtgggagtt tattactgcg tgcaaggtag acatctgcac	360
acgttcggtc aaggggaccag gctggagata aaacgaactg tggcagcacc aagcgtcttc	420
atcttccgc 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> 285

<211> 1404

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 285

atggaagccc cagcgcagct tctcttcctc ctgctactct ggctccctga taccaccgga	60

Sequence Listing

gaggtacagc tgggtggagtc gggaggaggc ttggtacagc ctggggggttc tctgagactc	120
tcctgtgcag cttctggcctt cacattcacc gactattaca tgagctgggt ccgccaggct	180
ccagggaagg gacttgagtg ggtgggtttc ataaggaaca aggctaacgg ttatacaacc	240
gagtacaacg cttccgttaa aggccgggttc accatctcca gagatgattc caaaaacagc	300
ctctatcttc aaatgaattc cctgaaaact gaagacactg ccgtttatta ctgtaccagg	360
tatatctttt tcgattactg gggccaaggc accactgtca cagtctcctc agcctccacc	420
aaggggcccat cgggtcttccc cctggcaccc tcctccaaga gcacctctgg gggtagacgc	480
gccctgggct gcctgggtcaa ggactacttc cccgaaccgg tgacgggtgtc gtggaactca	540
ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac	600
tccctcagca gcgtgggtgac cgtgccctcc agcagcttgg gcaccagac ctacatctgc	660
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt	720
gacaaaactc acacatgccc accgtgcccga gcacctgaac tcctggggggg accgtcagtc	780
ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca	840
tgcgtgggtg tggacgtgag ccacgaagac cctgagggtca agttcaactg gtacgtggac	900
ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac	960
cgtgtgggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag	1020
tgcaaggctc ccaacaaagc cctcccagcc cccatcgaga aaaccatctc caaagccaaa	1080
gggcagcccc gagaaccaca ggtgtacacc ctgcccccat cccgggatga gctgaccaag	1140
aaccagggtca gcctgacatg cctgggtcaaa ggcttctatc ccagcgacat cgccgtggag	1200
tgggagagca atgggcagcc ggagaacaac tacaagacca cgctcccgt gctggactcc	1260
gacggctcct tcttctctta cagcaagctc accgtggaca agtccagggtg gcagcagggg	1320
aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc	1380
ctctccctgt ctccgggtaa atga	1404

<210> 286

<211> 720

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 286

atggaagccc cagcgcagct tctcttctc ctgctactct ggctccctga taccaccgga	60
gatattgtaa tgacccaaac acccctctct ctttcagtca cacctggaca gccagcgtcc	120
atctcctgca ggtcctcaca gagtctctgt cacaccaatg gcaattccta cctgcattgg	180
tacctgcaga agcccgggca gagccccag ttgctgatct atatgggtgtc taatcggttc	240
tccggagtcc ccgacagatt ttctggttca gggctctggaa ctgattttac actgaagatt	300

Sequence Listing

agtcgggtcg aggccgagga tgtaggcgtg tattactgct caciaagcac acatgtgccg	360
ttcacttttcg gccaaaggaac aaagctcgaa atcaagcgaa ctgtggcagc accaagcgctc	420
ttcatcttcc cgccatctga tgagcagttg aaatctggaa ctgcctctgt tgtgtgcctg	480
ctgaataact tctatcccag agaggccaaa gtacagtggg aggtggataa cgccctccaa	540
tcgggtaact cccaggagag tgtcacagag caggacagca aggacagcac ctacagcctc	600
agcagcacc tgacgctgag caaagcagac tacgagaaac acaaagtcta cgcttgcgaa	660
gtcaccatc agggcctgag ctcgcccgtc acaaagagct tcaacagggg agagtgttag	720

<210> 287

<211> 1395

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<220>

<221> modified_base

<222> (1398)..(1398)

<223> a, c, t, g, unknown or other

<400> 287

atggaagccc cagcgcagct tctcttctc ctgctactct ggctccctga taccaccgga	60
gaggtacagc ttcttgaaag tggaggtggc cttgtccaac ccggagggtc attgcggttg	120
agctgtgcgg caagtggctt caccttctct gacgcttggg tggactgggt gagacaagcc	180
cccggtaagg gactggagtt ggtttctgaa atcaggaaca agccaagaa ccatgcaaca	240
tattatgccg aaagtgtgaa gggaaggttc acaatcagta gagataacag caagaacaca	300
ctgtacctcc agatgaacag cctcagagct gaggacaccg ccgtctatta ttgtaccact	360
ctcgattcat gggggcaggg taccaccgtt acagtcagca gcgcctccac caagggccca	420
tcggtcttcc ccctggcacc ctctccaag agcacctctg ggggtacagc ggccctgggc	480
tgcttggtca aggactactt ccccgaaccg gtgacggtgt cgtggaactc aggcgccttg	540
accagcggcg tgcacacctt cccggctgtc ctacagtcct caggactcta ctccctcagc	600
agcgtggtga ccgtgccctc cagcagcttg ggcaccaga cctacatctg caacgtgaat	660
cacaagccca gcaacaccaa ggtggacaag aaagttgagc ccaaattctg tgacaaaact	720
cacacatgcc caccgtgcc agcacctgaa ctctggggg gaccgtcagt cttctcttc	780
ccccaaaac ccaaggacac cctcatgatc tcccggacc ctgaggtcac atgcgtggtg	840
gtggacgtga gccacgaaga ccctgaggtc aagttcaact ggtacgtgga cggcgtggag	900
gtgcataatg ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtggtc	960
agcgtcctca ccgtcctgca ccaggactgg ctgaatggca aggagtacaa gtgcaaggctc	1020
tccaacaaag ccctcccagc ccccatcgag aaaaccatct ccaaagccaa agggcagccc	1080
cgagaaccac aggtgtacac cctgccccca tcccgggatg agctgaccaa gaaccaggctc	1140

Sequence Listing

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agcctgacat gcctgggtcaa aggcttctat cccagcgaca tcgccgtgga gtgggagagc      1200
aatgggcagc cggagaacaa ctacaagacc acgcctcccg tgctggactc cgacgggtcc      1260
ttcttctctt acagcaagct caccgtggac aagtccaggt ggcagcaggg gaacgtcttc      1320
tcatgctccg tgatgcatga ggctctgcac aaccactaca cgcagaagag cctctccctg      1380
tctccgggta aatga                                                    1395

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<210> 288

<211> 1395

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<220>

<221> modified_base

<222> (1398)..(1398)

<223> a, c, t, g, unknown or other

<400> 288

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atggaagccc cagcgcagct tctcttcctc ctgctactct ggctccctga taccaccgga      60
gaggtacagc ttcttgaaag tggaggtggc cttgtccaac ccggaggggc attgcggttg      120
agctgtgcgg caagtggctt caccttctct gacgcttgga tggactgggt gagacaagcc      180
cccggtaagg gactggagtt ggttgctgaa atcaggaaca aggccaagaa ccatgcaaca      240
tattatgccg aaagtgtgaa gggaagggtc acaatcagta gagataacag caagaacaca      300
ctgtacctcc agatgaacag cctcagagct gaggacaccg ccgtctatta ttgtaccact      360
ctcgattcat gggggcaggg taccaccgtt acagtcagca ggcctccac caagggccca      420
tcggtcttcc ccctggcacc ctctccaag agcacctctg ggggtacagc ggccctgggc      480
tgcttggtca aggactactt ccccgaaccg gtgacggtgt cgtggaactc aggcgcctgt      540
accagcggcg tgcacacctt cccggctgtc ctacagtcct caggactcta ctccctcagc      600
agcgtggtga ccgtgccctc cagcagcttg ggcaccaga cctacatctg caacgtgaat      660
cacaagccca gcaacaccaa ggtggacaag aaagttgagc ccaaattctg tgacaaaact      720
cacacatgcc caccgtgccc agcacctgaa ctctggggg gaccgtcagt cttcctcttc      780
ccccaaaac ccaaggacac cctcatgatc tcccggacct ctgaggtcac atgcgtggtg      840
gtggacgtga gccacgaaga ccctgaggtc aagttcaact ggtacgtgga cggcgtggag      900
gtgcataatg ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtggtc      960
agcgtcctca ccgtcctgca ccaggactgg ctgaatggca aggagtacaa gtgcaaggtc     1020
tccaacaaag ccctcccagc ccccatcgag aaaaccatct ccaaagccaa agggcagccc     1080
cgagaaccac aggtgtacac cctgccccca tcccgggatg agctgaccaa gaaccaggtc     1140
agcctgacat gcctgggtcaa aggcttctat cccagcgaca tcgccgtgga gtgggagagc     1200

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Sequence Listing

aatgggcagc cggagaacaa ctacaagacc acgcctcccg tgctggactc cgacggctcc	1260
ttcttcctct acagcaagct caccgtggac aagtccaggt ggcagcaggg gaacgtcttc	1320
tcatgctccg tgatgcatga ggctctgcac aaccactaca cgcagaagag cctctccctg	1380
tctccgggta aatga	1395

<210> 289

<211> 720

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 289

atggaagccc cagcgcagct tctcttcctc ctgctactct ggctccctga taccaccgga	60
gatatcgtga tgacacaaac tcccctgtct ctgtctgtaa ctccaggtca gcccgcgagt	120
atttcatgta agagcagcca atccctgctg gacagcgacg ggaagaccta cctgaactgg	180
ttactccaaa agccaggaca aagtcccaa cgccttattt acctggtgtc agccctggac	240
tctggcgtgc ccgatcgatt tagcggcagc gggagtggca cagatttcac cctgaaaata	300
tcccgcgtcg aggccgaaga tgtgggcgtg tactactgct ggcagggcac acatttcccc	360
tggacatttg gtcaggggac aaagctggaa attaaacgaa ctgtggcagc accaagcgtc	420
ttcatcttcc cgccatctga tgagcagttg aaatctggaa ctgcctctgt tgtgtgcctg	480
ctgaataact tctatcccag agaggccaaa gtacagtgga aggtggataa cgccctccaa	540
tcgggtaact cccaggagag tgtcacagag caggacagca aggacagcac ctacagcctc	600
agcagcacc tgacgctgag caaagcagac tacgagaaac acaaagtcta cgcctgcgaa	660
gtcaccatc agggcctgag ctgcccgtc acaaagagct tcaacagggg agagtgttag	720

<210> 290

<211> 1395

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 290

atggaagccc cagcgcagct tctcttcctc ctgctactct ggctccctga taccaccgga	60
gaggtacagc tgggtggagtc gggaggaggc ttggtacagc ctgggggttc tctgagactc	120
tcctgtgcag cttctggatt ccattcagt aactactgga tgaactgggt ccgccaggct	180
ccagggaagg gacttgagt ggtgggtcaa attagattga aatctaataa ttatgcaaca	240
cattatgcgg agtctgtgaa agggcggttc accatctcca gagatgattc caaaaacagc	300
ctctatcttc aaatgaattc cctgaaaact gaagacactg ccgtttatta ctgtacccca	360
attgactatt ggggccaagg caccactgtc acagtctcct cagcctccac caagggccca	420

Sequence Listing

tcggtcttcc ccctggcacc ctctccaag agcacctctg ggggtacagc ggccctgggc	480
tgcttggtca aggactactt ccccgaaccg gtgacggtgt cgtggaactc aggcgccttg	540
accagcggcg tgcacacett cccggctgtc ctacagtcct caggactcta ctccctcagc	600
agcgtggtga ccgtgccctc cagcagcttg ggcacccaga cctacatctg caacgtgaat	660
cacaagccca gcaacaccaa ggtggacaag aaagttgagc ccaaattcttg tgacaaaact	720
cacacatgcc caccgtgccc agcacctgaa ctcttggggg gaccgtcagt ctctctcttc	780
cccccaaaac ccaaggacac cctcatgata tcccggaccc ctgaggtcac atgcgtggtg	840
gtggacgtga gccacgaaga ccctgaggtc aagttcaact ggtacgtgga cggcgtggag	900
gtgcataatg ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtggtc	960
agcgtcctca ccgtcctgca ccaggactgg ctgaatggca aggagtacaa gtgcaaggtc	1020
tccaacaaag ccctcccagc ccccatcgag aaaaccatct ccaaagccaa agggcagccc	1080
cgagaaccac aggtgtacac cctgccccca tcccgggatg agctgaccaa gaaccaggtc	1140
agcctgacat gcctggtcaa aggtttctat cccagcgaca tcgccgtgga gtgggagagc	1200
aatgggcagc cggagaacaa ctacaagacc acgcctcccg tgctggactc cgacggctcc	1260
ttcttctct acagcaagct caccgtggac aagtccaggt ggcagcaggg gaacgtcttc	1320
tcatgtccg tgatgcatga ggctctgcac aaccactaca cgagaagag cctctccctg	1380
tctccgggta aatga	1395

<210> 291

<211> 717

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 291

atggaagccc cagcgcagct tctcttctc ctgctactct ggctccctga taccaccgga	60
gacattgtga tgaccagac tccactcagt ttgtcagtta cccctgggca accagcctct	120
atctcttgca agtcaagtca gagcctctta tatagtaatg gaaaaaccta tttgaactgg	180
gttttacaga agccaggcca gtctccacag cgcctaactt atctggtgtc taaactggac	240
tctggagtcc ctgacagggt ctctggcagt ggatcaggaa cagattttac actgaaaatc	300
agcagagtgg aggctgagga tgtgggagtt tattactgag tgcaagggtc acattttcac	360
acgttcggtc aagggaacaa gctggagatt aaacgaactg tggcagcacc aagcgtcttc	420
atcttcccg catctgatga gcagttgaaa tctggaactg cctctgttgt gtgcctgctg	480
aataacttct atcccagaga ggccaaagta cagtgggaagg tggataacgc cctccaatcg	540
ggtaactccc aggagagtgt cacagagcag gacagcaagg acagcaccta cagcctcagc	600
agcaccctga cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc	660
acccatcagg gcctgagctc gcccgtcaca aagagcttca acaggggaga gtgttag	717

Sequence Listing

<210> 292
 <211> 1404
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic polynucleotide

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<400> 292
atggaagccc cagcgcagct tctcttcctc ctgctactct ggctccctga taccaccgga      60
gaggtacagc tgggtggagtc gggaggaggc ttggtacagc ctggggggttc tctgagactc      120
tcctgtgcag cttctggatt caccttttct gattactaca tgagctgggt ccgccaggct      180
ccagggaagg gacttgagtg ggtgggtttt attagaaaca aagctaattg ttacacaaca      240
gagtacagtg catctgtgaa gggtcggttc accatctcca gagatgattc caaaaacagc      300
ctctatcttc aaatgaattc cctgaaaact gaagacactg ccgtttatta ctgtgcaaga      360
tatatctggg ttgactactg gggccaaggc accactgtca cagtctcttc agcctccacc      420
aagggcccat cggctcttccc cctggcaccc tcctccaaga gcacctctgg gggtagacgc      480
gccctgggct gcctgggtcaa ggactacttc cccgaaccgg tgacgggtgtc gtggaactca      540
ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcttc aggactctac      600
tcctcagca gcgtgggtgac cgtgccctcc agcagcttgg gcaccagac ctacatctgc      660
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt      720
gacaaaactc acacatgccc accgtgcccga gcacctgaac tcctgggggg accgtcagtc      780
ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca      840
tgcgtgggtg tggacgtgag ccacgaagac cctgagggtca agttcaactg gtacgtggac      900
ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac      960
cgtgtgggtc gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag     1020
tgcaagggtc ccaacaaagc cctcccagcc cccatcgaga aaaccatctc caaagccaaa     1080
gggcagcccc gagaaccaca ggtgtacacc ctgcccccat cccgggatga gctgaccaag     1140
aaccagggtc gcctgacatg cctgggtcaa ggcttctatc ccagcgacat cgccgtggag     1200
tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc     1260
gacggctcct tcttctcta cagcaagctc accgtggaca agtccagggt gcagcagggg     1320
aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc     1380
ctctccctgt ctccgggtaa atga                                             1404
    
```

<210> 293
 <211> 723
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic

polynucleotide

Sequence Listing

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<400> 293
atggaagccc cagcgagct tctcttcctc ctgctactct ggctccctga taccaccgga      60
gacattgtga tgacccaaac tccactctcc ctgtctgtca ctctggaca accagcctcc      120
atctcttgca gatctagtca gagccttgta cacactaatg gaaacaccta ttacattgg      180
tacctgcaga agccaggcca gtctccacag ctctgattt atatggtttc caaccgattt      240
tctgggggtcc cagacagggt cagtggcagt ggatcaggga cagatttcac actcaagatc      300
agcagagtgg aggctgagga tgtgggagtt tattactgct ctcaaagtgc acatgttcct      360
ccgctcacgt tcggtaagg gaccaggctg gagattaaac gaactgtggc agcaccaagc      420
gtcttcattc tcccgccatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc      480
ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgccctc      540
caatcgggta actcccagga gagtgtcaca gacaggaca gcaaggacag cacctacagc      600
ctcagcagca ccctgacgct gagcaaagca gactacgaga aacacaaagt ctacgcctgc      660
gaagtcaccc atcagggcct gagctcgccc gtcacaaaga gcttcaacag gggagagtgt      720
tag                                                                    723

```

```

<210> 294
<211> 8
<212> PRT
<213> Artificial Sequence

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<220>
<223> Description of Artificial Sequence: Synthetic
      peptide

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<400> 294
Ala Arg Tyr Ile Phe Phe Asp Tyr
1           5

```