

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
10 June 2010 (10.06.2010)

PCT

(10) International Publication Number
WO 2010/065962 A2

(51) International Patent Classification:

C07K 16/30 (2006.01) **C12N 15/13** (2006.01)
C07K 19/00 (2006.01) **A61P 35/00** (2006.01)
A61K 39/395 (2006.01)

(21) International Application Number:

PCT/US2009/067017

(22) International Filing Date:

7 December 2009 (07.12.2009)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/120,269 5 December 2008 (05.12.2008) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report (Rule 48.2(g))

— with sequence listing part of description (Rule 5.2(a))

(54) Title: MONOCLONAL ANTIBODIES FOR USE IN DIAGNOSIS AND THERAPY OF CANCERS AND AUTOIMMUNE DISEASE

(57) Abstract: The specification describes the sequences for antibodies that recognize the HLA-A2- restricted peptide PR-I in the context of HLA presentation on the surface of cancer cells. Use of these antibodies in the diagnosis and treatment of cancer and immune-related diseases are also provided.



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DESCRIPTION**MONOCLONAL ANTIBODIES FOR USE IN DIAGNOSIS AND THERAPY OF
CANCERS AND AUTOIMMUNE DISEASE**

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BACKGROUND OF THE INVENTION

10 This application claims benefit of priority to U.S. Provisional Application Serial No. 61/120,269, filed December 5, 2008, the entire contents of which are hereby incorporated by reference.

This invention was made with government support under P50 CA100632 awarded by the National Cancer Institute/National Institutes of Health. The government has certain rights in the invention.

15 **1. Field of the Invention**

The present invention relates generally to the fields of cancer and immunotherapy. More particularly, it concerns immunodiagnostic and immunotherapeutic antibodies for the treatment and prevention of cancer and autoimmune disease.

20 **2. Description of Related Art**

The immune system has long been implicated in the control of cancer; however, evidence for specific and efficacious immune responses in human cancer have been lacking. In chronic myelogenous leukemia (CML), either allogeneic bone marrow transplant (BMT) or interferon- α 2b (IFN- α 2b) therapy have resulted in complete remission, but the mechanism
25 for disease control is unknown and may involve immune antileukemic responses.

Based on evidence in the art, it is thought that lymphocytes play a role in mediating an antileukemia effect. Studies have demonstrated that allogeneic donor lymphocyte infusions (DLI) have been used to treat relapse of myeloid leukemia after allogeneic BMT (Giralt and Kolb, 1996; Kolb and Holler, 1997; Kolb *et al.*, 1995; Kolb *et al.*, 1996; Antin,
30 1993). Lymphocyte transfusion from the original bone marrow (BM) donor induces both hematological and cytogenetic responses in approximately 70% to 80% of patients with chronic myelocytic leukemia (CML) in chronic phase (CP) (Kolb *et al.*, 1996, Kolb and Holler, 1997). Remissions after DLI for AML are generally not as durable as those obtained

in chronic phase CML, which may reflect the rapid kinetics of tumor growth outpacing the kinetics of the developing immune response. Additionally, most patients with myeloid forms of leukemia will die from the disease unless they can be treated with allogeneic bone marrow transplant, where the associated graft versus leukemia (GVL) effect cures patients. However, graft-versus-host disease (GVHD) and transplant-related toxicity limit this treatment. It is believed that GVL may be separable from GVHD, and that targeting the immune response toward leukemia-associated antigens will allow for the transfer of GVL to patients without GVHD.

Thus, if more antigens (*i.e.*, leukemia antigens or antigens against other cancers) could be determined, and if large numbers of the most potent antigen-specific cytotoxic T lymphocytes (CTLs) could be obtained, it would allow for development of leukemia-specific therapies, breast cancer specific therapies, *etc.* using the antigens as targets for vaccines or for generating specific T-cells for use in adoptive immunotherapy.

PR1, an HLA-A2.1-restricted nonamer derived from proteinase 3 (P3) and elastase, was identified as a leukemia-associated antigen (Molldrem *et al.*, 2000; Molldrem *et al.*, 1996; Molldrem *et al.*, 1997; Molldrem *et al.*, 1999; Molldrem *et al.*, 2003 each incorporated herein by reference in their entirety). The finding that PR1 is a leukemia-associated antigen has been independently confirmed by Burchert *et al.* (2002) and Scheibenbogen *et al.* (2002). CTLs that are specific for PR1 kill AML, CML and MDS cells, but not normal bone marrow cells. In a recent phase I/II vaccine study, the PR1 peptide has been administered to patients with AML, CML and MDS, and PR1-specific CTL immunity has been elicited in 47% of patients, and clinical responses have been observed in 26%. Thus, this antigen provides an interesting platform for further investigation into anti-cancer immune responses as well as for the development of new therapeutic agents.

SUMMARY OF THE INVENTION

Thus, in accordance with the present invention, there is provided an isolated and purified antibody that binds to VLQELNVTV (SEQ ID NO:1) when bound by an HLA-A2 receptor. The heavy chain variable region CDR1-3 segments may comprise SEQ ID NOS:3, 5 and 7, and the entire heavy chain variable region sequence comprises SEQ ID NO:9 or 25. The light chain variable region CDR1-3 segments may comprise SEQ ID NOS:15, 19 and 23, and the entire heavy chain variable region sequence comprises SEQ ID NO:24. The antibody

may be a single chain antibody and/or may be fused to a non-antibody peptide or polypeptide segment. The antibody may be linked to a diagnostic reagent, such as a diagnostic reagent is a fluorophore, a chromophore, a dye, a radioisotope, a chemilluminant molecule, a paramagnetic ion, or a spin-trapping reagent. The antibody may be linked to a therapeutic reagent, such as a cytokine, a chemotherapeutic, a radiotherapeutic, a hormone, an antibody Fc fragment, a TLR agonist, a CpG-containing molecule, or an immune co-stimulatory molecule. The antibody may be a mouse antibody or may be a humanized antibody.

In another embodiment, the present invention provided a nucleic acid encoding the heavy chain CDRs of SEQ ID NOS:3, 5 and/or 7. The nucleic acid may encode a heavy chain variable region of SEQ ID NO:9 or 25. The nucleic acid may further comprise a nucleic acid segment encoding a non-T-cell peptide or polypeptide. Also provided, is a nucleic acid encoding the light chain CDRs of SEQ ID NOS:15, 19 and/or 23. The nucleic acid may encode a heavy chain variable region of SEQ ID NO:9 or 25. The nucleic acid may also further comprise a promoter sequence positioned 5' to the nucleic acid encoding the heavy/light chain CDRs, such as a promoter active in eukaryotic cells or prokaryotic cells. The nucleic acid may be located in a replicable vector, such as a non-viral vector or a viral vector. The nucleic acid may further comprise linker-encoding segments, wherein said linker-encoding segments located between said CDR-encoding segments. One or more of the linker-encoding segments may encode a helix-turn-helix motif.

In yet another embodiment, there is provided an artificial antibody comprising a heavy chain-encoding segment comprising CDRs comprising the sequences of SEQ ID NO:3, 5 and 7. In still another embodiment, there is provided an artificial antibody comprising a light chain-encoding segment comprising CDRs comprising the sequences of SEQ ID NO:15, 19 and 23. The CDRs may be joined by synthetic linkers. The said heavy chain may be fused to a non-antibody peptide or polypeptide segment. The antibody may be linked to a diagnostic reagent, such as a fluorophore, a chromophore, a dye, a radioisotope, a chemilluminant molecule, a paramagnetic ion, or a spin-trapping reagent. The antibody may be linked to a therapeutic reagent, such as a cytokine, a toxin, a chemotherapeutic, a radiotherapeutic, a hormone, an antibody Fc fragment, a TLR agonist, neutrophil elastase, proteinase 3, a CpG-containing molecule, or an immune co-stimulatory molecule. Also provided is a nucleic acid encoding a heavy chain-encoding segment comprising CDRs comprising the sequences of SEQ ID NO:3, 5 and 7, or or a light chain-encoding segment comprising CDRs comprising the sequences of SEQ ID NO:15, 19 and 23.

In still yet another embodiment, there is provided a method of making an antibody comprising (a) introducing into a host cell (i) a nucleic acid sequence encoding a heavy chain comprising SEQ ID NOS:3, 5 and 7, and (ii) a nucleic acid sequence encoding a light chain comprising SEQ ID NOS:15, 19 and 23; and (b) culturing said host cell under conditions supporting expression of said light and heavy chains. The heavy chain variable region may comprise SEQ ID NO:9 or 25 and the light chain variable region may comprising SEQ ID NO:24. The method may further comprise the step of linking said antibody to a diagnostic or therapeutic agent.

Another embodiment of the present invention provides a method of detecting abnormal cells in a sample suspected of containing abnormal cells comprising contacting said sample with an antibody or artificial antibody as described above. The antibody or artificial antibody may be conjugated to a diagnostic agent, such as a fluorophore, a chromophore, a dye, a radioisotope, a chemilluminiscent molecule, a paramagnetic ion, or a spin-trapping reagent. The antibody or artificial antibody may be detected using a secondary binding agent, such as an anti-Fc receptor antibody. The sample may be (a) a tumor tissue from head & neck, brain, esophagus, breast, lung, liver, spleen, stomach, small intestine, large intestine, rectum, ovary, uterus, cervix, prostate, testicle or skin tissue, or (b) a fluid such as blood, lymph, urine, bone marrow aspirate or nipple aspirate. The sample may be from a resected tumor bed. The method may further comprise making a treatment decision based on the presence, absence or degree of detection, such as deciding to treat said subject with a PR-1-based peptide vaccine. The method may detect primary cancer cells, metastatic cancer cells or myeloid dysplastic cells are detected.

In still another embodiment, there is provided a method of treating a subject with cancer comprising administering to said subject an antibody or artificial antibody as described above. The antibody or artificial antibody may be conjugated to a therapeutic agent, such a cytokine, a toxin, a chemotherapeutic, a radiotherapeutic, a hormone, an antibody Fc fragment, a TLR agonist, a CpG-containing molecule, or an immune co-stimulatory molecule. The cancer may be a solid tumor, such as a head & neck tumor, a brain tumor, an esophageal tumor, a breast tumor, a lung tumor, a liver tumor, a spleen tumor, and stomach tumor, a small intestinal tumor, a large intestinal tumor, a rectal tumor, an ovarian tumor, a uterine tumor, a cervical tumor, a prostate tumor, a testicular tumor or a skin tumor. Alternatively, the cancer may be a blood cancer, such as a leukemia or lymphoma. The cancer may be recurrent or metastatic cancer. The method may further comprise providing said subject with a second anti-cancer therapy, such as a gene therapy, a chemotherapy, a radiotherapy, a

hormone therapy, a toxin therapy or surgery. The antibody or artificial antibody may be administered to said subject more than once.

In yet a further embodiment, there is provided a method of treating a subject with an autoimmune disease comprising administering to said subject an antibody or artificial antibody as described above. The autoimmune disease may be Wegener's granulomatosis, Churg-Strauss Syndrome, or systemic small vessel vasculitis. The antibody or artificial antibody may be conjugated to a therapeutic agent, such as a toxin or apoptosis-inducing agent. The method may further comprise providing said subject with a second anti-autoimmune therapy, such as an anti-inflammatory agent. The antibody may be administered to said subject more than once.

Yet additional embodiments included (i) a purified and isolated nucleic acid segment encoding a CDR comprising the amino sequence of SEQ ID NO:3, such as where the nucleotide sequence comprises SEQ ID NO:2; (ii) a purified and isolated nucleic acid segment encoding a CDR comprising the amino sequence of SEQ ID NO:5, such as where the nucleotide sequence comprises SEQ ID NO:4; (iii) a purified and isolated nucleic acid segment encoding a CDR comprising the amino sequence of SEQ ID NO:7, such as where the nucleotide sequence comprises SEQ ID NO:6; (iv) a purified and isolated nucleic acid segment encoding a heavy chain variable region comprising the amino sequence of SEQ ID NO:9, such as where the nucleotide sequence comprises SEQ ID NO:8; (v) a purified and isolated nucleic acid segment encoding a CDR comprising the amino sequence of SEQ ID NO:15, such as where the nucleotide sequence comprises SEQ ID NO:14; (vi) a purified and isolated nucleic acid segment encoding a CDR comprising the amino sequence of SEQ ID NO:19, such as where the nucleotide sequence comprises SEQ ID NO:18; (vii) a purified and isolated nucleic acid segment encoding a CDR/JK comprising the amino sequence of SEQ ID NO:23, such as where the nucleotide sequence comprises SEQ ID NO:22; and (viii) a purified and isolated nucleic acid segment encoding a heavy chain variable region comprising the amino sequence of SEQ ID NO:24, such as where the nucleotide sequence comprises the first 324 bases of SEQ ID NO:10.

Additional methods include (i) treating a subject with a myeloid dysplastic disease comprising administering to said subject the antibody or artificial antibody described above; and (ii) inducing complement-mediated cytotoxicity of an HLA-A2 cancer cell comprising contacting said cancer cell with the antibody or artificial antibody described above.

As used herein the specification, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising," the words "a" or "an"

may mean one or more than one. As used herein "another" may mean at least a second or more.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1 - Specificity of 8F4 for PR1/HLA-A2. ELISA with recombinant peptide/HLA-A2 monomers, loaded with PR1 or single amino acid-modified PR1 analogs. To determine the amino acid positions within the PR1 sequence that are essential for optimal 8F4 binding, HLA-A2 monomers loaded with peptides containing alanine substitutions in PR1 (ALA1-ALA9) coated onto microtiter wells at increasing concentrations. Wells were then incubated with a fixed concentration of 8F4 or the control antibody bb7.2 (an HLA-A*0201 allele-specific mouse IgG2a monoclonal antibody). Binding was measured by ELISA using peroxidase-conjugated goat anti-mouse antibody. 8F4 bound to HLA-A2 loaded with PR1 and to most of PR1 analogs, with significantly less binding to the ALA1 analogue (alanine substituted for valine in position 1 of the peptide), and no binding to control peptide pp65/HLA-A2. Control antibody bb7.2 bound equally well to PR1- and pp65-loaded HLA-A2.

FIG. 2 - Affinity of 8F4 monoclonal antibody to PR1/HLA-A2. Affinity measurements of peptide/HLA-A2 binding to 8F4 and bb7.2 antibodies was determined by surface plasmon resonance using the BIAcore 3000. The test antibodies were captured onto anti-mouse antibody-coated surfaces. The analyte, peptide/HLA-A2, was diluted to 100 nM and tested in duplicate for binding to the antibody-coated surfaces. The analysis was performed at 25C using PBS, 0.005% Tween-20, 0.1 mg/ml BSA, pH 7.4 as the

running buffer. To obtain the binding affinity the experimental data were fit to a first-order kinetic model (shown as orange lines in the figures) and K_D for 8F4 and bb7.2 was subsequently determined.

FIG. 3 - Specificity of 8F4 for HLA-A2+ AML. Multiparameter flow cytometry of leukemia and normal PBMC with 8F4 and cell surface antibodies. PBMC from AML patients and normal donors were gated on live cells using aqua and then stained with 8F4 (conjugated with ALEXA Fluor 647), bb7.2 (conjugated with FITC), and surface phenotype antibodies for CD13 and CD33, and analyzed by flow cytometry. The following gating strategy was used: first, aqua- live cells were analyzed for CD13 and CD33 expression, and double positive cells were analyzed for expression of PR1/HLA-A2 (8F4) and total HLA-A2 expression (bb7.2). Negative quadrant gating was established using HLA-A2-negative AML control cells.

FIGS. 4A-B - 8F4 Antibody induces complement-dependent cytotoxicity (CDC) of AML. Target cells were washed and resuspended in 10-RPMI/HEPES at a concentration 5×10^5 cells/ml. Twenty microliter (μ l) of antibody and 100 μ l of cells were mixed and warmed to 37°C in 96-well plates, then 20 μ l of ice cold standard rabbit complement (Cedarlane, Ontario, Canada) was added and incubated at 37°C for 90 min. The cytotoxicity was determined using the Cyto-Tox Glo Cytotoxicity Assay (Promega). The antibody-specific CDC (AB-CDC) was calculated as: $AB-CDC = ((L_{C+AB} - L_{C-AB}) / (L_{max} - L_S)) \times 100\%$, where L_{C+AB} is target cell lysis in the presence of complement plus antibody; L_{T+C} is lysis in the presence of complement alone; L_{spont} and L_{max} were measured before and after adding the cytotoxic agent digitonin to the cells, per the manufacturers instructions. (FIG. 4A) Incubation with 20 μ g 8F4 induced complement-dependent cytotoxicity of PBMC and leukapheresis (LP) cells taken from HLA-A2+ AML patients, but did not lyse control samples of PBMC from HLA-A2-negative AML or PBMC from HLA-A2+ normal donor. (FIG. 4B) 8F4-mediated lysis of HLA-A2+ AML was antibody dose-dependent, whereas isotype control antibody (IgG2a mouse anti-KLH) and human intravenous immunoglobulin (commercial IVIG) showed no lysis of AML.

FIG. 5 - Specificity of 8F4 for AML But Not Normal PBMC. Surface staining of AML, PBMC, and T2 cell for PR1 and HLA-A2. Cells were stained with anti-PR1/HLA-A2 antibody (8F4)-alexa-647 (red) and anti-HLA-A2-FITC conjugated (green), fixed with 1% paraformaldehyde, and then studied using confocal microscopy. T2 cells were pulsed with PR1 peptide (20 μ g/ml) as a positive control and with the CMV peptide pp65 (20

μg/ml) as a negative control peptide. PR1/HLA-A2 expression is evident on the cell surface of AML and PR1-pulsed T2 cells, but not on HLA-A2+ PBMC or on the pp65-pulsed T2 cells. Dapi-blue was used for nuclear staining.

FIG. 6 - 8F4 antibody prevents engraftment of AML in *in vivo* model. Primary HLA-A2+ leukemia cells (10^6) were washed, resuspended in PBS (100 μl), mixed with 8F4 or isotype control antibody (20 μg) and intravenously injected into 200 cGy-irradiated HLA-A2+ transgenic NOD/SCID mice. After two weeks mice were sacrificed, dissected, and the tissues were homogenized and analyzed for the presence of leukemia cells by flow cytometry with human and mouse cell surface markers. Flow cytometry results of cells isolated from mouse bone marrow (BM) are shown. Control (PBS-treated) and experimental animals that received AML cells plus 8F4 (AML+ 8F4 antibody) showed no human leukemia cell in BM. In contrast, animals that received AML cells plus control antibody (AML+ isotype control) showed human CD33+CD45+ cells in bone marrow, with the same phenotype as the infused AML.

FIG. 7 - Immunization Strategy to Obtain Anti-PR1/HLA-A2 Antibody. Schematic representation of MHC class I molecule. MHC class consists of heavy chain and a β2 microglobulin chain. Peptide antigen binds into the groove of the MHC-I, flanked by α1 and α2 helical domains of the chain.

FIGS. 8A-B - 8F4 Antibody Prevents Engraftment of Human AML in HLA-A2 Tg Xenograft Model. Primary HLA-A2+ AML cells (10^6) were washed, resuspended in PBS (250 μl), mixed with 20 μg 8F4 or isotype control antibody and intravenously injected into sub-lethally irradiated (200 cGy) HLA-A2 Tg NOD/SCID mice. At the indicated times, peripheral blood, bone marrow and tissues were analyzed for presence of leukemia by histochemistry (FIG. 8A) and flow cytometry (FIG. 8B). Irradiated mice without AML transfer and pre-transfer AML cells were used as negative and positive controls, respectively. (FIG. 8A) AML infiltration in tissues of experimental mice following injection with AML cells plus 8F4 (left panels), injection with AML cells plus isotype control antibody (iso, central panels), and no AML transfer control mice (right panels). (FIG. 8B) AML cells (shown pre-transfer, left panels) were not detected in the bone marrow (top two panels) and peripheral blood (bottom two panels) of no transfer control and experimental 8F4-treated mice. Mice that received AML cells mixed with isotype matched control antibody (iso) showed engraftment of AML1 and AML5 two or four weeks after AML transfer. An extended panel, including a mouse cell specific

marker (mCD45), 3-6 human markers (CD45, CD13, CD33, CD34, CD38, HLA-DR), and Live/Dead Fixable Aqua (Invitrogen) was used for flow cytometric analysis of AML engraftment. All plots show viable mCD45- cells.

FIGS. 9A-C - 8F4 Induces Transient (21-day) Neutropenia in HLA-A2 Transgenic NOD/SCID due to the Expression of Conserved PR1 Sequence on HLA-A2-Expressing Murine Hematopoietic Cells. HLA-A2 Tg NOD/SCID mice were injected with 200 μ g (10 mg/kg) 8F4 or isotype control Ab. These animals have been shown to present endogenous PR1. Nine days later, bone marrow cells were harvested and stained with mAb directed to mouse antigens (B220-PE, Gr-1-PB, CD11b-APC, F4/80-PE-Cy7, CD3-FITC and LIVE/DEAD Fixable Aqua) and examined by flow cytometry. (FIG. 9A) Reduced granulocytes were evident in scatter profiles of bone marrow (left panels). Gr-1lo immature neutrophils were present, but Gr-1hi mature neutrophils were less numerous in the bone marrow of 8F4-treated mice (center panels). Additionally, monocytes (SSC^{lo} CD11b⁺; lower right gate of right panels) were reduced in 8F4-treated animals. (FIG. 9B) Intravenous injection of 8F4 (5 mg/kg) induced transient reduction in absolute numbers of circulating mature granulocytes, macrophages and monocytes in HLA-A2 Tg NOD/SCID mice. Three weeks after treatment all populations remain. Gates shown in FIG. 9A were used to determine the frequency of each cell type as a percentage of live cells. Error bars are standard deviations of n = 2 animals per group. One representative experiment out of three shown. (FIG. 9C) No significant pathological changes were evident in liver, lung, spleen, kidney, heart or brains of HLA-A2 Tg NOD/SCID mice 7 days after injection of 200 μ g (10 mg/kg) 8F4. H&E sections of representative tissues from 2 mice are shown.

FIGS. 10A-B - 8F4 Induces Transient Leukopenia of Established Human Hematopoietic Cells after Transfer of Human CD34⁺ Cell Enriched Cord Blood into NOD/SCID Mice. Fresh HLA-A2⁺ cord blood (CB) units (50-150 ml) were ficolled by using Histopaque1077, washed with PBS, then with CliniMACS buffer (0.5% HSA in PBS pH 7.2/1 mM EDTA, Miltenyi). 10⁸ cells were resuspended in 300 ml MACS buffer, mixed with 100 ml CD34 Microbeads (Miltenyi) and incubated at 4°C for 30 minutes and washed. CD34⁺ cells, labeled with magnetic beads, were purified by using 2 LS columns (Miltenyi). CD34⁺ cells were eluted from the column, counted and iv injected into the irradiated (400 rad) NOD/SCID mouse (1-2.5 x 10⁶ cells per mouse). Control mouse CB1-5 did not received CB cells. (FIG. 10A) Beginning 4 weeks after transplant,

peripheral blood from mice was taken weekly or every other week to monitor cord blood engraftment by using FACS with mouse CD45, human CD45, and HLA markers. 9-12 weeks after transfer mice were i.v. injected with 20 μ g (1 mg/kg) 8F4 twice with one week interval between injection (dotted arrows). (FIG. 10B) Four weeks after 2nd 8F4 injection mice were sacrificed. Blood, spleen and bone marrow were analyzed for engraftment of human cells as above.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

I. The Present Invention

The PR-1 self-peptide (VLQELNVTV; SEQ ID NO:1) has been shown to be recognized on leukemia cell membrane-expressed HLA-A*0201 by CD8⁺ cytotoxic T lymphocytes (CTL), and PR1-specific CTL specifically lyse myeloid leukemia but not normal bone marrow cells. Vaccination of HLA-A2⁺ patients with AML, CML, and MDS with PR1 peptide induced PR-1-CTL immunity in 58% of patients and objective clinical responses in 13 of 66 (20%) patients. While these results are encouraging, high tumor burden remains a barrier to successful vaccination.

Because PR1 peptide is expressed in sufficient quantity only on the surface of myeloid leukemia cells and not on normal bone marrow cells, the inventors sought to develop an antibody targeted to PR1/HLA-A*0201 that might be used therapeutically to treat patients with myeloid leukemia or that could be used to identify which patients might be susceptible to PR1-based immunotherapy, such as vaccines or adoptive T-cell transfer. Since HLA-A2⁺ is the most commonly expressed HLA allele (40% of the general Caucasian population), antibody-based therapy for a T-cell epitope therefore would be novel and it might be widely applied. By immunizing immune competent BALB/c mice with recombinant PR1/HLA-A*0201 monomers, they obtained an IgG2a-kappa monoclonal antibody (8F4) with specificity for the combined PR1/HLA-A*0201 epitope. The 8F4 antibody was shown to have high affinity for PR1/HLA-A*0201 (K_D = 9.9 nanomolar) and it was shown to only recognize PR1-pulsed T2 target cells but not control peptide-pulsed cells. 8F4 binds to HLA-A2⁺ AML using both FACS and confocal microscopy to label the cells, but not to normal HLA-A2⁺ peripheral blood cells.

In addition, 8F4 induced dose-dependent complement-mediated cytotoxicity (CDC) of HLA-A2⁺ primary human leukemia but not normal bone marrow cells. Significantly, 8F4 antibody specifically prevented engraftment of human AML in an HLA-A2 transgenic

NOD/SCID animal model with only a single exposure to antibody at the time of adoptive transfer into the animal. Taken together, these results show that the creation of an antibody with specificity for the cell membrane-bound PR1/HLA-A*0201 epitope, an important T-cell target antigen, that specifically targets and eliminates primary human leukemia in an animal model.

II. Definitions

The phrases “isolated” or “biologically pure” refer to material which is substantially or essentially free from components which normally accompany the material as it is found in its native state. Thus, isolated peptides in accordance with the invention preferably do not contain materials normally associated with the peptides in their *in situ* environment.

“Major histocompatibility complex” or “MHC” is a cluster of genes that plays a role in control of the cellular interactions responsible for physiologic immune responses. In humans, the MHC complex is also known as the HLA complex. For a detailed description of the MHC and HLA complexes, see Paul (1993).

“Human leukocyte antigen” or “HLA” is a human class I or class II major histocompatibility complex (MHC) protein (see, *e.g.*, Stites, 1994).

An “HLA supertype or family,” as used herein, describes sets of HLA molecules grouped on the basis of shared peptide-binding specificities. HLA class I molecules that share somewhat similar binding affinity for peptides bearing certain amino acid motifs are grouped into HLA superotypes. The terms HLA superfamily, HLA supertype family, HLA family, and HLA xx-like supertype molecules (where xx denotes a particular HLA type), are synonyms.

The term “motif” refers to the pattern of residues in a peptide of defined length, usually a peptide of from about 8 to about 13 amino acids for a class I HLA motif and from about 6 to about 25 amino acids for a class II HLA motif, which is recognized by a particular HLA molecule. Peptide motifs are typically different for each protein encoded by each human HLA allele and differ in the pattern of the primary and secondary anchor residues.

“Abnormal cell” is any cell that is considered to have a characteristic a typical for that cell type, including atypical growth, typical growth in an atypical location or typical action against an atypical target. Such cells include cancer cells, benign hyperplastic or dysplastic cells, inflammatory cells or autoimmune cells.

III. PR-1 and HLA Restriction

A. PR-1

The PR-1 self-peptide (VLQELNVTV; SEQ ID NO:1) is derived from proteinase 3 (P3) and neutrophil elastase (NE), both aberrantly expressed in leukemia. It has been shown to be recognized on leukemia cell membrane-expressed HLA-A*0201 by CD8⁺ cytotoxic T lymphocytes (CTL). PR-1-specific CTL specifically lyse myeloid leukemia, including acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), and myelodysplastic syndrome (MDS) but not normal bone marrow cells. Previously, the inventor has shown that PR-1 vaccination of HLA-A2⁺ patients with AML, CML, and MDS with PR1 peptide in Montanide ISA-51 and GM-CSF induced PR-1-CTL immunity in 58% of patients and objective clinical responses in 13 of 66 (20%) patients.

B. HLA-A2

The human leukocyte antigen system (HLA) is the name of the major histocompatibility complex (MHC) in humans. The superlocus contains a large number of genes related to immune system function in humans. This group of genes resides on chromosome 6, and encode cell-surface antigen-presenting proteins and many other genes. The proteins encoded by certain genes are also known as antigens, as a result of their historic discovery as factors in organ transplantations. The major HLA antigens are essential elements in immune function. Different classes have different functions.

HLA class I antigens (A, B & C) present peptides from inside the cell (including viral peptides if present). These peptides are produced from digested proteins that are broken down in the lysosomes. The peptides are generally small polymers, about 9 amino acids in length. Foreign antigens attract killer T-cells (also called CD8⁺ cells) that destroy cells. HLA class II antigens (DR, DP & DQ) present antigens from outside of the cell to T-lymphocytes. These particular antigens stimulate T-helper cells to reproduce and these T-helper cells then stimulate antibody producing B-cells, self-antigens are suppressed by suppressor T-cells.

HLA-A2 (A2) is a human leukocyte antigen serotype within the HLA-A "A" serotype group. The serotype identifies the gene products of many HLA-A*02 alleles, including HLA-A*0201, *0202, *0203, *0206, and *0207 gene products. A*02 is globally common, but A*0201 is at high frequencies in Northern Asia and North America. A2 is the most diverse serotype, showing diversity in Eastern Africa and Southwest Asia. While the frequency of A*0201 in Northern Asia is high, its diversity is limited to A*0201 the less common Asian variants A*0203, A*0206.

The serotype is determined by the antibody recognition of α^2 subset of HLA-A α -chains. For A2, the α “A” chain are encoded by the HLA-A*02 allele group and the β -chain are encoded by B2M locus. A2 and A*02 are almost synonymous in meaning. A2 is more common in Northern Asia and North America than elsewhere, and it is part of a several long haplotypes.

IV. Antibodies

The present invention concerns the production and use of antibodies that bind to PR1 in the context of HLA-A2 presentation. Antibodies are capable of “specific binding” to a particular target or series of antigenically related targets. As used herein, an antibody is said to be capable of “specific binding” to a antigen if it discriminates from antigenically distinct molecules based on binding to the variable region of the antibody. Such interactions are in contrast to non-specific binding that involve classes of compounds, irrespective of their chemical structure (such as the binding of proteins to nitrocellulose, *etc.*). In particular, an antibody of the present invention can exhibit “highly specific binding” such that they will be incapable or substantially incapable of binding to even closely related molecules

Monoclonal antibodies can be readily prepared through use of well-known techniques such as those exemplified in U.S. Patent 4,196,265, herein incorporated by reference. Typically, a technique involves first immunizing a suitable animal with a selected antigen (*e.g.*, a polypeptide or polynucleotide of the present invention) in a manner sufficient to provide an immune response. Rodents such as mice and rats are preferred animals. Spleen cells from the immunized animal are then fused with cells of an immortal myeloma cell. Successful fusions are then screened for production of appropriate antibodies.

In one embodiment, antibody molecules will comprise fragments (such as (F(ab'), F(ab')₂) that are produced, for example, by the proteolytic cleavage of the mAbs, or single-chain immunoglobulins producible, for example, via recombinant means. Such antibody derivatives are monovalent. In one embodiment, such fragments can be combined with one another, or with other antibody fragments or receptor ligands to form “chimeric” binding molecules. Significantly, such chimeric molecules may contain substituents capable of binding to different epitopes of the same molecule, or they may be capable of binding to an activated protein C epitope and a “non-activated protein C” epitope.

Where the antibodies or their fragments are intended for therapeutic purposes, it may be desirable to “humanize” them in order to attenuate any immune reaction. Such humanized

antibodies may be studied in an *in vitro* or an *in vivo* context. Humanized antibodies may be produced, for example by replacing an immunogenic portion of an antibody with a corresponding, but non-immunogenic portion (*i.e.*, chimeric antibodies). PCT Application PCT/U.S.86/02269; EP Application 184,187; EP Application 171,496; EP Application 173,494; PCT Application WO 86/01533; EP Application 125,023; Sun *et al.* (1987); Wood *et al.* (1985); and Shaw *et al.* (1988); all of which references are incorporated herein by reference. General reviews of “humanized” chimeric antibodies are provided by Morrison (1985; also incorporated herein by reference. “Humanized” antibodies can alternatively be produced by CDR or CEA substitution. Jones *et al.* (1986); Verhoeyan *et al.* (1988); Beidler *et al.* (1988); all of which references are incorporated herein by reference.

A. Variants

The following is a discussion based upon changing the amino acids of a protein to create a modified protein. In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

It also is understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: basic amino acids: arginine (+3.0), lysine (+3.0), and histidine (-0.5); acidic amino acids: aspartate (+3.0 $\pm$ 1), glutamate (+3.0 $\pm$ 1), asparagine (+0.2), and glutamine (+0.2); hydrophilic, nonionic amino acids: serine (+0.3), asparagine (+0.2), glutamine (+0.2), and threonine (-0.4), sulfur containing amino acids: cysteine (-1.0) and methionine (-1.3); hydrophobic, nonaromatic amino acids: valine (-1.5), leucine (-1.8), isoleucine (-1.8), proline (-0.5 $\pm$ 1), alanine (-0.5), and glycine (0); hydrophobic, aromatic amino acids: tryptophan (-3.4), phenylalanine (-2.5), and tyrosine (-2.3).

It is understood that an amino acid can be substituted for another having a similar hydrophilicity and produce a biologically or immunologically modified protein. In such

changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those that are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

As outlined above, amino acid substitutions generally are based on the relative
5 similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take into consideration the various foregoing characteristics are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

10 The present invention may also employ the use of peptide mimetics for the preparation of polypeptides (*see e.g.*, Johnson, 1993) having many of the natural properties of an antibody, but with altered and/or improved characteristics. The underlying rationale behind the use of mimetics is that the peptide backbone of proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions, such as those of
15 antibody and antigen. These principles may be used, in conjunction with the principles outline above, to engineer second generation molecules having many of the natural properties of an antibody but with altered and even improved characteristics.

It is contemplated that the present invention may further employ sequence variants such as insertional or deletion variants. Deletion variants lack one or more residues of the
20 native protein. Insertional mutants typically involve the addition of material at a non-terminal point in the polypeptide. It also will be understood that insertional sequence variants may include N- or C-terminal amino acids, and yet still be essentially as set forth in one of the sequences disclosed herein, so long as the sequence meets the criteria set forth above, including the maintenance of biological activity.

25 The present invention also contemplate isotype modification. As discussed below, antibody 8F4 was determined to be an IgG2a- κ . By modifying the Fc region to have a different isotype, different functionalities can be achieved. For example, changing to IgG1 can increase antibody dependent cell cytotoxicity, switching to class A can improve tissue distribution, and switching to class M can improve valency.

30 Modified antibodies may be made by any technique known to those of skill in the art, including expression through standard molecular biological techniques, or the chemical synthesis of polypeptides. Methods for recombinant expression are addressed elsewhere in this document.

B. Single Chain Antibody

A Single Chain Variable Fragment (scFv) is a fusion of the variable regions of the heavy and light chains of immunoglobulins, linked together with a short (usually serine, glycine) linker. This chimeric molecule retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of a linker peptide. The image to the right shows how this modification usually leaves the specificity unaltered. These molecules were created historically to facilitate phage display where it is highly convenient to express the antigen binding domain as a single peptide. Alternatively, scFv can be created directly from subcloned heavy and light chains derived from a hybridoma. Single chain variable fragments lack the constant Fc region found in complete antibody molecules, and thus, the common binding sites (*e.g.*, protein A/G) used to purify antibodies. These fragments can often be purified/immobilized using Protein L since Protein L interacts with the variable region of kappa light chains.

Flexible linkers generally are comprised of helix- and turn-promoting amino acid residues such as alanine, serine and glycine. However, other residues can function as well. Tang *et al.* (1996) used phage display as a means of rapidly selecting tailored linkers for single-chain antibodies (scFvs) from protein linker libraries. A random linker library was constructed in which the genes for the heavy and light chain variable domains were linked by a segment encoding an 18-amino acid polypeptide of variable composition. The scFv repertoire (approx. 5×10^6 different members) was displayed on filamentous phage and subjected to affinity selection with hapten. The population of selected variants exhibited significant increases in binding activity but retained considerable sequence diversity. Screening 1054 individual variants subsequently yielded a catalytically active scFv that was produced efficiently in soluble form. Sequence analysis revealed a conserved proline in the linker two residues after the V_H C terminus and an abundance of arginines and prolines at other positions as the only common features of the selected tethers.

The recombinant antibodies of the present invention may also involve sequences or moieties that permit dimerization or multimerization of the receptors. Such sequences include those derived from IgA, which permit formation of multimers in conjunction with the J-chain. Another multimerization domain is the Gal4 dimerization domain. In other embodiments, the chains may be modified with agents such as biotin/avidin, which permit the combination of two antibodies.

In a separate embodiment, a single-chain antibody can be created by joining receptor light and heavy chains using a non-peptide linker or chemical unit. Generally, the light and heavy chains will be produced in distinct cells, purified, and subsequently linked together in an appropriate fashion (*i.e.*, the N-terminus of the heavy chain being attached to the C-terminus of the light chain via an appropriate chemical bridge).

Cross-linking reagents are used to form molecular bridges that tie functional groups of two different molecules, *e.g.*, a stabilizing and coagulating agent. However, it is contemplated that dimers or multimers of the same analog or heteromeric complexes comprised of different analogs can be created. To link two different compounds in a step-wise manner, hetero-bifunctional cross-linkers can be used that eliminate unwanted homopolymer formation. Table 3 illustrates several cross-linkers.

TABLE 3 - HETERO-BIFUNCTIONAL CROSS-LINKERS

linker	Reactive Toward	Advantages and Applications	Spacer Arm Length\after cross-linking
SMPT	Primary amines Sulfhydryls	· Greater stability	11.2 Å
SPDP	Primary amines Sulfhydryls	· Thiolation · Cleavable cross-linking	6.8 Å
LC-SPDP	Primary amines Sulfhydryls	· Extended spacer arm	15.6 Å
Sulfo-LC-SPDP	Primary amines Sulfhydryls	· Extended spacer arm · Water-soluble	15.6 Å
SMCC	Primary amines Sulfhydryls	· Stable maleimide reactive group · Enzyme-antibody conjugation · Hapten-carrier protein conjugation	11.6 Å
Sulfo-SMCC	Primary amines Sulfhydryls	· Stable maleimide reactive group · Water-soluble · Enzyme-antibody conjugation	11.6 Å
MBS	Primary amines Sulfhydryls	· Enzyme-antibody conjugation · Hapten-carrier protein conjugation	9.9 Å
Sulfo-MBS	Primary amines Sulfhydryls	· Water-soluble	9.9 Å
SIAB	Primary amines Sulfhydryls	· Enzyme-antibody conjugation	10.6 Å
Sulfo-SIAB	Primary amines Sulfhydryls	· Water-soluble	10.6 Å
SMPB	Primary amines Sulfhydryls	· Extended spacer arm · Enzyme-antibody conjugation	14.5 Å
Sulfo-SMPB	Primary amines Sulfhydryls	· Extended spacer arm · Water-soluble	14.5 Å
EDC/Sulfo-NHS	Primary amines Carboxyl groups	· Hapten-Carrier conjugation	0
ABH	Carbohydrates Nonselective	· Reacts with sugar groups	11.9 Å

An exemplary hetero-bifunctional cross-linker contains two reactive groups: one reacting with primary amine group (*e.g.*, N-hydroxy succinimide) and the other reacting with a thiol group (*e.g.*, pyridyl disulfide, maleimides, halogens, *etc.*). Through the primary amine reactive group, the cross-linker may react with the lysine residue(s) of one protein (*e.g.*, the selected antibody or fragment) and through the thiol reactive group, the cross-linker, already tied up to the first protein, reacts with the cysteine residue (free sulfhydryl group) of the other protein (*e.g.*, the selective agent).

It is preferred that a cross-linker having reasonable stability in blood will be employed. Numerous types of disulfide-bond containing linkers are known that can be successfully employed to conjugate targeting and therapeutic/preventative agents. Linkers that contain a disulfide bond that is sterically hindered may prove to give greater stability *in vivo*, preventing release of the targeting peptide prior to reaching the site of action. These linkers are thus one group of linking agents.

Another cross-linking reagent is SMPT, which is a bifunctional cross-linker containing a disulfide bond that is "sterically hindered" by an adjacent benzene ring and methyl groups. It is believed that steric hindrance of the disulfide bond serves a function of protecting the bond from attack by thiolate anions such as glutathione which can be present in tissues and blood, and thereby help in preventing decoupling of the conjugate prior to the delivery of the attached agent to the target site.

The SMPT cross-linking reagent, as with many other known cross-linking reagents, lends the ability to cross-link functional groups such as the SH of cysteine or primary amines (*e.g.*, the epsilon amino group of lysine). Another possible type of cross-linker includes the hetero-bifunctional photoreactive phenylazides containing a cleavable disulfide bond such as sulfosuccinimidyl-2-(p-azido salicylamido) ethyl-1,3'-dithiopropionate. The N-hydroxy-succinimidyl group reacts with primary amino groups and the phenylazide (upon photolysis) reacts non-selectively with any amino acid residue.

In addition to hindered cross-linkers, non-hindered linkers also can be employed in accordance herewith. Other useful cross-linkers, not considered to contain or generate a protected disulfide, include SATA, SPDP and 2-iminothiolane (Wawrzynczak & Thorpe, 1987). The use of such cross-linkers is well understood in the art. Another embodiment involves the use of flexible linkers.

U.S. Patent 4,680,338, describes bifunctional linkers useful for producing conjugates of ligands with amine-containing polymers and/or proteins, especially for forming antibody conjugates with chelators, drugs, enzymes, detectable labels and the like. U.S. Patents

5,141,648 and 5,563,250 disclose cleavable conjugates containing a labile bond that is cleavable under a variety of mild conditions. This linker is particularly useful in that the agent of interest may be bonded directly to the linker, with cleavage resulting in release of the active agent. Particular uses include adding a free amino or free sulfhydryl group to a protein, such as an antibody, or a drug.

U.S. Patent 5,856,456 provides peptide linkers for use in connecting polypeptide constituents to make fusion proteins, *e.g.*, single chain antibodies. The linker is up to about 50 amino acids in length, contains at least one occurrence of a charged amino acid (preferably arginine or lysine) followed by a proline, and is characterized by greater stability and reduced aggregation. U.S. Patent 5,880,270 discloses aminooxy-containing linkers useful in a variety of immunodiagnostic and separative techniques.

C. Purification

In certain embodiments, the antibodies of the present invention may be purified. The term “purified,” as used herein, is intended to refer to a composition, isolatable from other components, wherein the protein is purified to any degree relative to its naturally-obtainable state. A purified protein therefore also refers to a protein, free from the environment in which it may naturally occur. Where the term “substantially purified” is used, this designation will refer to a composition in which the protein or peptide forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 90%, about 95% or more of the proteins in the composition.

Protein purification techniques are well known to those of skill in the art. These techniques involve, at one level, the crude fractionation of the cellular milieu to polypeptide and non-polypeptide fractions. Having separated the polypeptide from other proteins, the polypeptide of interest may be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity). Analytical methods particularly suited to the preparation of a pure peptide are ion-exchange chromatography, exclusion chromatography; polyacrylamide gel electrophoresis; isoelectric focusing. Other methods for protein purification include, precipitation with ammonium sulfate, PEG, antibodies and the like or by heat denaturation, followed by centrifugation; gel filtration, reverse phase, hydroxylapatite and affinity chromatography; and combinations of such and other techniques.

In purifying an antibody of the present invention, it may be desirable to express the polypeptide in a prokaryotic or eukaryotic expression system and extract the protein using

denaturing conditions. The polypeptide may be purified from other cellular components using an affinity column, which binds to a tagged portion of the polypeptide. As is generally known in the art, it is believed that the order of conducting the various purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified protein or peptide.

Commonly, complete antibodies are fractionated utilizing agents (*i.e.*, protein A) that bind the Fc portion of the antibody. Alternatively, antigens may be used to simultaneously purify and select appropriate antibodies. Such methods often utilize the selection agent bound to a support, such as a column, filter or bead. The antibodies is bound to a support, contaminants removed (*e.g.*, washed away), and the antibodies released by applying conditions (salt, heat, *etc.*).

Various methods for quantifying the degree of purification of the protein or peptide will be known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific activity of an active fraction, or assessing the amount of polypeptides within a fraction by SDS/PAGE analysis. Another method for assessing the purity of a fraction is to calculate the specific activity of the fraction, to compare it to the specific activity of the initial extract, and to thus calculate the degree of purity. The actual units used to represent the amount of activity will, of course, be dependent upon the particular assay technique chosen to follow the purification and whether or not the expressed protein or peptide exhibits a detectable activity.

It is known that the migration of a polypeptide can vary, sometimes significantly, with different conditions of SDS/PAGE (Capaldi *et al.*, 1977). It will therefore be appreciated that under differing electrophoresis conditions, the apparent molecular weights of purified or partially purified expression products may vary.

D. Conjugation of Antibodies to Therapeutic or Diagnostic Agents

In one embodiment, the antibodies of the present invention may be linked to various reagents for use in diagnosis and therapy of disease. Linking may be performed using a variety of well known chemical reactions and agents, some of which are described elsewhere in this document.

1. Diagnostic Reagents

Many diagnostic/imaging agents are known in the art, as are methods for their attachment to proteins, including antibodies (see, *e.g.*, U.S. Patents 5,021,236; 4,938,948; and

4,472,509, each incorporated herein by reference). The imaging moieties used can be paramagnetic ions, radioactive isotopes, fluorochromes, NMR-detectable substances, and X-ray imaging agents.

In the case of paramagnetic ions, one might mention by way of example ions such as chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) and/or erbium (III), with gadolinium being particularly preferred. Ions useful in other contexts, such as X-ray imaging, include but are not limited to lanthanum (III), gold (III), lead (II), and especially bismuth (III).

In the case of radioactive isotopes for therapeutic and/or diagnostic application, one might mention astatine²¹¹, ¹⁴carbon, ⁵¹chromium, ³⁶chlorine, ⁵⁷cobalt, ⁵⁸cobalt, copper⁶⁷, ¹⁵²Eu, gallium⁶⁷, ³hydrogen, iodine¹²³, iodine¹²⁵, iodine¹³¹, indium¹¹¹, ⁵⁹iron, ³²phosphorus, rhenium¹⁸⁶, rhenium¹⁸⁸, ⁷⁵selenium, ³⁵sulphur, technetium^{99m} and/or yttrium⁹⁰. ¹²⁵I is often being preferred for use in certain embodiments, and technetium^{99m} and/or indium¹¹¹ are also often preferred due to their low energy and suitability for long range detection. Radioactively labeled receptors of the present invention may be produced according to well-known methods in the art. For instance, receptors can be iodinated by contact with sodium and/or potassium iodide and a chemical oxidizing agent such as sodium hypochlorite, or an enzymatic oxidizing agent, such as lactoperoxidase. TcRs according to the invention may be labeled with technetium^{99m} by ligand exchange process, for example, by reducing pertechnetate with stannous solution, chelating the reduced technetium onto a Sephadex column and applying the antibody to this column. Alternatively, direct labeling techniques may be used, *e.g.*, by incubating pertechnetate, a reducing agent such as SnCl_2 , a buffer solution such as sodium-potassium phthalate solution, and the antibody. Intermediary functional groups which are often used to bind radioisotopes, which exist as metallic ions to antibody are diethylenetriaminepentaacetic acid (DTPA) or ethylene diaminetetracetic acid (EDTA).

Among the fluorescent labels contemplated for use as conjugates are Alexa 350, Alexa 430, AMCA, BODIPY 630/650, BODIPY 650/665, BODIPY-FL, BODIPY-R6G, BODIPY-TMR, BODIPY-TRX, Cascade Blue, Cy3, Cy5,6-FAM, Fluorescein Isothiocyanate, HEX, 6-JOE, Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, REG, Rhodamine Green, Rhodamine Red, Renographin, ROX, TAMRA, TET, Tetramethylrhodamine, and/or Texas Red.

2. Therapeutic Reagents

A wide variety of therapeutic agents made linked to antibodies of the present invention. For example, the radioisotopes discussed above, though useful in diagnostic contexts, may be also be used as therapeutic agents. Chemotherapeutics may also be
5 conjugated to antibodies, and include cisplatin (CDDP), carboplatin, procarbazine, mechlorethamine, cyclophosphamide, camptothecin, ifosfamide, melphalan, chlorambucil, busulfan, nitrosurea, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicomycin, mitomycin, etoposide (VP16), tamoxifen, raloxifene, estrogen receptor binding agents, taxol, gemcitabine, navelbine, farnesyl-protein transferase inhibitors, transplatinum, 5-fluorouracil,
10 vincristine, vinblastine and methotrexate.

Another class of therapeutic agent is the toxins. Cholera toxin, botulism toxin, pertussis toxin, ricin A and B chains, as well as other natural or synthetic toxins are contemplated.

Cytokines and lymphokines are yet another class of therapeutic agents than can be
15 coupled to the TcR of the present invention, and include IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, TNF α , GM-CSF, INF α , IFN β , and IFN γ .

In other embodiments, anti-inflammatory agents are contemplated as therapeutic agents that may be conjugated to antibodies. Anti-inflammatories include NSAIDs, steroids,
20 rapamycin, infliximab, and ontak. Immunosuppressive agents include FK-506 and cyclosporin A.

TLR agonist may be linked to the antibody, *e.g.*, through the Fc portion of the molecule. Agonists of TLRs are compounds that stimulate, or “turn on,” the immune system. Natural agonists for TLR9 are components of DNA that are common to bacteria and viruses.
25 Natural agonists for TLRs 7 and 8 are patterns of RNA found in viruses. Following recognition of their natural DNA and RNA agonists, TLRs 7, 8, and 9 each initiate a different cascade of protective immune responses. TLR agonists include oligodeoxynucleotides, hyaluronic acid fragments, imiquimod, lavendustin C, lipid A, loroxibine, LPS, monophosphoryl lipid A, myristicin, resiquimod, *S. typhimurium* flagellin, HKLM,
30 PAM3CSK4, and polyI:C.

IV. Nucleic Acids and Expression

A. Antibody Encoding Nucleic Acids

One aspect of the invention, nucleic acid are provided that encode various portions of antibody heavy and light chain, variable and constant domains. A nucleic acid segment may be derived from genomic DNA, complementary DNA (cDNA) or synthetic DNA. Where incorporation into an expression vector is desired, the nucleic acid may also comprise a natural intron or an intron derived from another gene, as well as other non-coding (*e.g.*, regulatory) and coding regions (*e.g.*, linkers). As used herein, the term “cDNA” is intended to refer to DNA prepared using messenger RNA (mRNA) as template. The advantage of using a cDNA, as opposed to genomic DNA or DNA polymerized from a genomic, non- or partially-processed RNA template, is that the cDNA primarily contains coding sequences of the corresponding protein.

The term “recombinant” may be used in conjunction with a polypeptide or the name of a specific polypeptide, and generally refers to a polypeptide produced from a nucleic acid molecule that has been manipulated *in vitro* or that is the replicated product of such a molecule. Recombinant vectors and isolated nucleic acid segments may variously include the antibody-coding regions themselves, coding regions bearing selected alterations or modifications in the basic coding region, or they may encode larger polypeptides that include non-antibody regions.

A “nucleic acid” as used herein includes single-stranded and double-stranded molecules, as well as DNA, RNA, chemically modified nucleic acids and nucleic acid analogs. It is contemplated that a nucleic acid within the scope of the present invention may be of about 10, about 20, about 30, about 40, about 50, about 60, about 70, about 80, about 90, about 100, about 110, about 120, about 130, about 140, about 150, about 160, about 170, about 180, about 190, about 200, about 210, about 220, about 230, about 240, about 250, about 275, about 300, about 325, about 350, about 375, about 400, about 425, about 450, about 475, about 500, about 525, about 550, about 575, about 600, about 625, about 650, about 675, about 700, about 725, about 750, about 775, about 800, about 825, about 850, about 875, about 900, about 925, about 950, about 975, about 1000, about 1100, about 1200, about 1300, about 1400, about 1500, about 1750, about 2000, about 2250, about 2500 or greater nucleotide residues in length.

It is contemplated that antibody may be encoded by any nucleic acid sequence that encodes the appropriate amino acid sequence, such as those in SEQ ID NOS: 3, 5, 7, 15, 19, 23 (heavy CDRs 1, 2 and 3; light CDRs 1 and 2, 3/JK), and SEQ ID NOS: 9 or 25, which

includes the heavy CDRs and framework regions 1, 2 and 3, which flank upstream of heavy CDRs 1, 2 and 3, respectively, and SEQ ID NO: 24, which includes the light CDRs and framework regions 1, 2 and 3, which flank upstream of light CDRs 1, 2 and 3, respectively. The design and production of nucleic acids encoding a desired amino acid sequence is well known to those of skill in the art, using standardized codon tables (Table 4). In particular embodiments, the codons selected for encoding each amino acid may be modified to optimize expression of the nucleic acid in the host cell of interest. The term “functionally equivalent codon” is used herein to refer to codons that encode the same amino acid, such as the six codons for arginine or serine, and also refers to codons that encode biologically equivalent amino acids. Codon preferences for various species of host cell are well known in the art. Codons preferred for use in humans, are well known to those of skill in the art (Wada *et al.*, 1990). Codon preferences for other organisms also are well known to those of skill in the art (Wada *et al.*, 1990, included herein in its entirety by reference).

Table 4 - Codon Table

Amino Acids			Codons						
Alanine	Ala	A	GCA	GCC	GCG	GCU			
Cysteine	Cys	C	UGC	UGU					
Aspartic acid	Asp	D	GAC	GAU					
Glutamic acid	Glu	E	GAA	GAG					
Phenylalanine	Phe	F	UUC	UUU					
Glycine	Gly	G	GGA	GGC	GGG	GGU			
Histidine	His	H	CAC	CAU					
Isoleucine	Ile	I	AUA	AUC	AUU				
Lysine	Lys	K	AAA	AAG					
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU	
Methionine	Met	M	AUG						
Asparagine	Asn	N	AAC	AAU					
Proline	Pro	P	CCA	CCC	CCG	CCU			
Glutamine	Gln	Q	CAA	CAG					
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU	
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU	
Threonine	Thr	T	ACA	ACC	ACG	ACU			
Valine	Val	V	GUA	GUC	GUG	GUU			

Amino Acids			Codons
Tryptophan	Trp	W	UGG
Tyrosine	Tyr	Y	UAC UAU

B. Nucleic Acid Expression

Prokaryote- and/or eukaryote-based systems can be used to produce nucleic acid sequences, or their cognate polypeptides, proteins and peptides. The present invention
5 contemplates the use of such an expression system to produce the antibodies that bind PR-1/HLA-A2. One powerful expression technology employs the insect-cell/baculovirus system. The insect-cell/baculovirus system can produce a high level of protein expression of a heterologous nucleic acid segment, such as described in U.S. Patents 5,871,986, 4,879,236,
both herein incorporated by reference, and which can be bought, for example, under the name
10 MAXBAC® 2.0 from INVITROGEN® and BACPACK™ BACULOVIRUS EXPRESSION SYSTEM FROM CLONTECH®.

In addition, numerous other expression systems exists which are commercially and widely available. One example of such a system is the STRATAGENE®'s COMPLETE CONTROL Inducible Mammalian Expression System, which involves a synthetic ecdysone-inducible
15 receptor, or its pET Expression System, an *E. coli* expression system. Another example of an inducible expression system is available from INVITROGEN®, which carries the T-REX™ (tetracycline-regulated expression) System, an inducible mammalian expression system that uses the full-length CMV promoter. INVITROGEN® also provides a yeast expression system called the *Pichia methanolica* Expression System, which is designed for high-level
20 production of recombinant proteins in the methylotrophic yeast *Pichia methanolica*. One of skill in the art would know how to express a vector, such as an expression construct, to produce a nucleic acid sequence or its cognate polypeptide, protein, or peptide.

1. Viral Vectors and Delivery

There are a number of ways in which expression vectors may be introduced into cells.
25 Viruses provide powerful tools for expression of protein products encoded by nucleic acids. Thus, in certain embodiments of the invention, the expression vector comprises a virus or engineered vector derived from a viral genome. The ability of certain viruses to enter cells *via* receptor-mediated endocytosis, to integrate into host cell genome and express viral genes stably and efficiently have made them attractive candidates for the transfer of foreign genes

into mammalian cells (Ridgeway, 1988; Nicolas and Rubenstein, 1988; Baichwal and Sugden, 1986; Temin, 1986). The first viruses used as gene vectors were DNA viruses including the papovaviruses (simian virus 40, bovine papilloma virus, and polyoma) (Ridgeway, 1988; Baichwal and Sugden, 1986) and adenoviruses (Ridgeway, 1988; Baichwal and Sugden, 1986).

Adenoviral Vectors. A particular method for delivery of the nucleic acid involves the use of an adenovirus expression vector. Although adenovirus vectors are known to have a low capacity for integration into genomic DNA, this feature is counterbalanced by the high efficiency of gene transfer afforded by these vectors. "Adenovirus expression vector" is meant to include those constructs containing adenovirus sequences sufficient to (a) support packaging of the construct and (b) to ultimately express a tissue or cell-specific construct that has been cloned therein. Knowledge of the genetic organization of adenovirus, a 36 kb, linear, double-stranded DNA virus, allows substitution of large pieces of adenoviral DNA with foreign sequences up to 7 kb (Grunhaus and Horwitz, 1992).

AAV Vectors. The nucleic acid may be introduced into the cell using adenovirus assisted transfection. Increased transfection efficiencies have been reported in cell systems using adenovirus coupled systems (Kelleher and Vos, 1994; Cotten *et al.*, 1992; Curiel, 1994). Adeno-associated virus (AAV) is an attractive vector system for use in the vaccines of the present invention (Muzyczka, 1992). AAV has a broad host range for infectivity (Tratschin *et al.*, 1984; Laughlin *et al.*, 1986; Lebkowski *et al.*, 1988; McLaughlin *et al.*, 1988). Details concerning the generation and use of rAAV vectors are described in U.S. Patents 5,139,941 and 4,797,368, each incorporated herein by reference.

Retroviral Vectors. Retroviruses have promise as gene delivery vectors in vaccines due to their ability to integrate their genes into the host genome, transferring a large amount of foreign genetic material, infecting a broad spectrum of species and cell types and of being packaged in special cell-lines (Miller, 1992).

In order to construct a retroviral vector, a nucleic acid (*e.g.*, one encoding an antigen of interest) is inserted into the viral genome in the place of certain viral sequences to produce a virus that is replication-defective. In order to produce virions, a packaging cell line containing the gag, pol, and env genes but without the LTR and packaging components is constructed (Mann *et al.*, 1983). When a recombinant plasmid containing a cDNA, together with the retroviral LTR and packaging sequences is introduced into a special cell line (*e.g.*, by calcium phosphate precipitation for example), the packaging sequence allows the RNA transcript of the recombinant plasmid to be packaged into viral particles, which are then

secreted into the culture media (Nicolas and Rubenstein, 1988; Temin, 1986; Mann *et al.*, 1983). The media containing the recombinant retroviruses is then collected, optionally concentrated, and used for gene transfer. Retroviral vectors are able to infect a broad variety of cell types. However, integration and stable expression require the division of host cells (Paskind *et al.*, 1975).

Lentiviruses are complex retroviruses, which, in addition to the common retroviral genes *gag*, *pol*, and *env*, contain other genes with regulatory or structural function. Lentiviral vectors are well known in the art (see, for example, Naldini *et al.*, 1996; Zufferey *et al.*, 1997; Blomer *et al.*, 1997; U.S. Patents 6,013,516 and 5,994,136). Some examples of lentivirus include the Human Immunodeficiency Viruses: HIV-1, HIV-2 and the Simian Immunodeficiency Virus: SIV. Lentiviral vectors have been generated by multiply attenuating the HIV virulence genes, for example, the genes *env*, *vif*, *vpr*, *vpu* and *nef* are deleted making the vector biologically safe.

Recombinant lentiviral vectors are capable of infecting non-dividing cells and can be used for both *in vivo* and *ex vivo* gene transfer and expression of nucleic acid sequences. For example, recombinant lentivirus capable of infecting a non-dividing cell wherein a suitable host cell is transfected with two or more vectors carrying the packaging functions, namely *gag*, *pol* and *env*, as well as *rev* and *tat* is described in U.S. Patent 5,994,136, incorporated herein by reference. One may target the recombinant virus by linkage of the envelope protein with an antibody or a particular ligand for targeting to a receptor of a particular cell-type. By inserting a sequence (including a regulatory region) of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell, for example, the vector is now target-specific.

Other Viral Vectors. Other viral vectors may be employed as vaccine constructs in the present invention. Vectors derived from viruses such as vaccinia virus (Ridgeway, 1988; Baichwal and Sugden, 1986; Coupar *et al.*, 1988), sindbis virus, cytomegalovirus and herpes simplex virus may be employed. They offer several attractive features for various mammalian cells (Friedmann, 1989; Ridgeway, 1988; Baichwal and Sugden, 1986; Coupar *et al.*, 1988; Horwich *et al.*, 1990). Lentiviruses also have been explored as vaccine vectors (VandenDriessche *et al.*, 2002).

Delivery Using Modified Viruses. A nucleic acid to be delivered may be housed within an infective virus that has been engineered to express a specific binding ligand. The virus particle will thus bind specifically to the cognate receptors of the target cell and deliver the contents to the cell. A novel approach designed to allow specific targeting of retrovirus

vectors was developed based on the chemical modification of a retrovirus by the chemical addition of lactose residues to the viral envelope. This modification can permit the specific infection of hepatocytes *via* sialoglycoprotein receptors.

Another approach to targeting of recombinant retroviruses was designed in which
5 biotinylated antibodies against a retroviral envelope protein and against a specific cell receptor were used. The antibodies were coupled *via* the biotin components by using streptavidin (Roux *et al.*, 1989). Using antibodies against major histocompatibility complex class I and class II antigens, they demonstrated the infection of a variety of human cells that bore those surface antigens with an ecotropic virus *in vitro* (Roux *et al.*, 1989).

10 2. Non-Viral Nucleic Acid Delivery

Suitable non-viral methods for nucleic acid delivery to effect expression of compositions of the present invention are believed to include virtually any method by which a nucleic acid (*e.g.*, DNA) can be introduced into an organelle, a cell, a tissue or an organism, as described herein or as would be known to one of ordinary skill in the art. Such methods
15 include, but are not limited to, direct delivery of DNA such as by injection (U.S. Patents 5,994,624, 5,981,274, 5,945,100, 5,780,448, 5,736,524, 5,702,932, 5,656,610, 5,589,466 and 5,580,859, each incorporated herein by reference), including microinjection (Harland and Weintraub, 1985; U.S. Patent 5,789,215, incorporated herein by reference); by electroporation (U.S. Patent 5,384,253, incorporated herein by reference); by calcium
20 phosphate precipitation (Graham and Van Der Eb, 1973; Chen and Okayama, 1987; Rippe *et al.*, 1990); by using DEAE-dextran followed by polyethylene glycol (Gopal, 1985); by direct sonic loading (Fechheimer *et al.*, 1987); by liposome mediated transfection (Nicolau and Sene, 1982; Fraley *et al.*, 1979; Nicolau *et al.*, 1987; Wong *et al.*, 1980; Kaneda *et al.*, 1989; Kato *et al.*, 1991); by microprojectile bombardment (PCT Application Nos. WO 94/09699
25 and 95/06128; U.S. Patents 5,610,042; 5,322,783 5,563,055, 5,550,318, 5,538,877 and 5,538,880, and each incorporated herein by reference); by agitation with silicon carbide fibers (Kaeppeler *et al.*, 1990; U.S. Patents 5,302,523 and 5,464,765, each incorporated herein by reference); or by PEG-mediated transformation of protoplasts (Omirulleh *et al.*, 1993; U.S. Patents 4,684,611 and 4,952,500, each incorporated herein by reference); by
30 desiccation/inhibition-mediated DNA uptake (Potrykus *et al.*, 1985). Through the application of techniques such as these, organelle(s), cell(s), tissue(s) or organism(s) may be stably or transiently transformed.

VI. Antibodies for Diagnosis of Cancer or Hyperplastic or Dysplastic Disorders

In an embodiment of the present invention, there are provided methods of diagnosing cancers such as leukemia (*e.g.*, AML, CML, MDS), as well as myelodysplastic disorders. Myelodysplasias (MDS) refer to a group of disorders in which the bone marrow does not function normally and produces insufficient number of normal blood cells. MDS affects the production of any, and occasionally all, types of blood cells including red blood cells, platelets, and white blood cells (cytopenias). About 50 %of pediatric myelodysplasia can be classified in five types of MDS: refractory anemia, refractory anemia with ring sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, and chronic myelomonocytic leukemia. The remaining 50% typically present with isolated or combined cytopenias such as anemia, leucopenia and/or thrombocytopenia (low platelet count). Although chronic, MDS progresses to become acute myeloid leukemia (AML) in about 30 percent of patients.

Also contemplated for diagnosis according to the present invention are solid tumor cancers. Such cancer lung cancer, head and neck cancer, breast cancer, pancreatic cancer, prostate cancer, renal cancer, bone cancer, testicular cancer, cervical cancer, gastrointestinal cancer, lymphomas, pre-neoplastic lesions in the lung, colon cancer, melanoma, and bladder cancer. Other hyperplastic, neoplastic and dysplastic diseases, including benign hyperproliferative diseases are also with the scope of the diagnostic procedures described herein.

A. Administration of Diagnostic Reagents

Adminstration of diagnostic reagents is well known in the art and will vary depending on diagnosis to be achieved. For example, where a discrete tumor mass or masses is/are to be imagined, local or regional administration (*e.g.*, in the the tumor vasculature, local lymph system or local arteries or veins) my be utilized. Alternatively, one may provide diagnostic reagents regionally or systemically. This may be the route of choice where imaging of an entire limb or organism is desired, where know specific tumor mass has been identified, or when metastasis is suspected.

B. Injectable Compositions and Formulations

One method for the delivery of a pharmaceutical according to the present invention is systemically. However, the pharmaceutical compositions disclosed herein may alternatively

be administered parenterally, intravenously, intradermally, intramuscularly, transdermally or even intraperitoneally as described in U.S. Patent 5,543,158; U.S. Patent 5,641,515 and U.S. Patent 5,399,363 (each specifically incorporated herein by reference in its entirety).

Injection of pharmaceuticals may be by syringe or any other method used for injection of a solution, as long as the agent can pass through the particular gauge of needle required for injection. A novel needleless injection system has been described (U.S. Patent 5,846,233) having a nozzle defining an ampule chamber for holding the solution and an energy device for pushing the solution out of the nozzle to the site of delivery. A syringe system has also been described for use in gene therapy that permits multiple injections of predetermined quantities of a solution precisely at any depth (U.S. Patent 5,846,225).

Solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (U.S. Patent 5,466,468, specifically incorporated herein by reference in its entirety). In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous,

intramuscular, subcutaneous, intratumoral and intraperitoneal administration. In this connection, sterile aqueous media that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermolysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The compositions disclosed herein may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like.

As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as

any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The phrase “pharmaceutically-acceptable” or “pharmacologically-acceptable” refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human. The preparation of an aqueous composition that contains a protein as an active ingredient is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared.

VII. Therapeutic Methods

A. Cancer and Hyperplastic/Dysplastic/Neoplastic Disease

The antibodies of the present invention may be used in the methods of treating hyperplastic/dysplastic/neoplastic diseases/conditions including cancer. Types of diseases/conditions contemplated to be treated with the peptides of the present invention include, but are not limited to leukemias such as, AML, MDS and CML, as well as myelodysplasias. Other types of cancers may include lung cancer, head and neck cancer, breast cancer, pancreatic cancer, prostate cancer, renal cancer, bone cancer, testicular cancer, cervical cancer, gastrointestinal cancer, lymphomas, pre-neoplastic lesions in the lung, colon cancer, melanoma, bladder cancer and any other neoplastic diseases.

To kill cells, inhibit-cell growth, inhibit metastasis, decrease tumor/tissue size, tumor cell burden or otherwise reverse or reduce the malignant phenotype of tumor cells, using the methods and compositions of the present invention, one would generally contact a hyperplastic/neoplastic/cancer cell with the therapeutic compound such as a polypeptide or an expression construct encoding an antibody of the present invention, normally dispersed in a pharmaceutically acceptable buffer or carrier (see above in the discussion of diagnostic agents). The routes of administration will vary, naturally, with the location and nature of the lesion, and include, *e.g.*, intradermal, transdermal, parenteral, intravenous, intramuscular, intranasal, subcutaneous, percutaneous, intratracheal, intraperitoneal, intratumoral, perfusion, lavage, direct injection, and oral administration and formulation. Any of the formulations and routes of administration discussed with respect to the treatment or diagnosis of cancer may also be employed with respect to neoplastic diseases and conditions. *Ex vivo* embodiments,

where tumor cells are treated/transduced outside a patient's body (either specifically or as part of a larger cell population) are contemplated.

Intratumoral injection, or injection into the tumor vasculature is specifically contemplated for discrete, solid, accessible tumors. Local, regional or systemic administration also may be appropriate. For tumors of >4 cm, the volume to be administered will be about 4-10 ml), while for tumors of <4 cm, a volume of about 1-3 ml will be used. Multiple injections delivered as single dose comprise about 0.1 to about 0.5 ml volumes. The viral particles may advantageously be contacted by administering multiple injections to the tumor, spaced at approximately 1 cm intervals.

In the case of surgical intervention, the present invention may be used may be used at the time of surgery, and/or thereafter, to treat residual or metastatic disease. For example, a resected tumor bed may be injected or perfused with a formulation comprising antibodies. The perfusion may be continued post-resection, for example, by leaving a catheter implanted at the site of the surgery. Periodic post-surgical treatment also is envisioned.

Continuous administration also may be applied where appropriate, for example, where a tumor is excised and the tumor bed is treated to eliminate residual, microscopic disease. Delivery *via* syringe or catheterization is preferred. Such continuous perfusion may take place for a period from about 1-2 hr, to about 2-6 hr, to about 6-12 hr, to about 12-24 hr, to about 1-2 days, to about 1-2 wk or longer following the initiation of treatment. Generally, the dose of the therapeutic composition *via* continuous perfusion will be equivalent to that given by a single or multiple injections, adjusted over a period of time during which the perfusion occurs. It is further contemplated that limb perfusion may be used to administer therapeutic compositions of the present invention, particularly in the treatment of melanomas and sarcomas.

Treatment regimens may vary as well, and often depend on tumor type, tumor location, disease progression, and health and age of the patient. Obviously, certain types of tumor will require more aggressive treatment, while at the same time, certain patients cannot tolerate more taxing protocols. The clinician will be best suited to make such decisions based on the known efficacy and toxicity (if any) of the therapeutic formulations.

In certain embodiments, the tumor being treated may not, at least initially, be resectable. Treatments may increase the resectability of the tumor due to shrinkage at the margins or by elimination of certain particularly invasive portions. Following treatments, resection may be possible. Additional treatments subsequent to resection will serve to eliminate microscopic residual disease at the tumor site.

A typical course of treatment, for a primary tumor or a post-excision tumor bed, will involve multiple doses. Typical primary tumor treatment involves a 6-dose application over a two-week period. The two-week regimen may be repeated one, two, three, four, five, six or more times. During a course of treatment, the need to complete the planned dosings may be re-evaluated.

B. Combination Therapies

It also may prove advantageous to use combination therapies, where a second anti-cancer agent is included. An “anti-cancer” agent is capable of negatively affecting cancer in a subject, for example, by killing cancer cells, inducing apoptosis in cancer cells, reducing the growth rate of cancer cells, reducing the incidence or number of metastases, reducing tumor size, inhibiting tumor growth, reducing the blood supply to a tumor or cancer cells, promoting an immune response against cancer cells or a tumor, preventing or inhibiting the progression of cancer, or increasing the lifespan of a subject with cancer. Anti-cancer agents include biological agents (biotherapy), chemotherapy agents, and radiotherapy agents. More generally, these other compositions would be provided with a therapy according to the present invention in a combined amount effective to kill or inhibit proliferation of the cell. This process may involve contacting the cells with the both agent(s) at the same time. This may be achieved by contacting the cell with a single composition or pharmacological formulation that includes both agents, or by contacting the cell with two distinct compositions or formulations at the same time.

Alternatively, the antibody therapy may precede or follow the other agent treatment by intervals ranging from minutes to weeks. In embodiments where the other agent and antibodies are applied separately to the cell, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the agent and expression construct would still be able to exert an advantageously combined effect on the cell. In such instances, it is contemplated that one may contact the cell with both modalities within about 12-24 h of each other and, more preferably, within about 6-12 h of each other. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

Various combinations may be employed; for example, the antibody therapy (with or without a conjugated therapeutic agent) is “A” and the secondary anti-cancer therapy is “B”:

A/B/A	B/A/B	B/B/A	A/A/B	A/B/B	B/A/A	A/B/B/B	B/A/B/B
B/B/B/A	B/B/A/B	A/A/B/B	A/B/A/B	A/B/B/A	B/B/A/A		
B/A/B/A	B/A/A/B	A/A/A/B	B/A/A/A	A/B/A/A	A/A/B/A		

5 Administration of the therapeutic agents of the present invention to a patient will follow general protocols for the administration of that particular secondary therapy, taking into account the toxicity, if any, of the antibody treatment. It is expected that the treatment cycles would be repeated as necessary. It also is contemplated that various standard therapies, as well as surgical intervention, may be applied in combination with the described cancer therapies.

1. Chemotherapy

Cancer therapies also include a variety of combination therapies with both chemical and radiation based treatments. Combination chemotherapies include, for example, cisplatin (CDDP), carboplatin, procarbazine, mechlorethamine, cyclophosphamide, camptothecin, ifosfamide, melphalan, chlorambucil, busulfan, nitrosurea, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicomycin, mitomycin, etoposide (VP16), tamoxifen, raloxifene, estrogen receptor binding agents, taxol, gemcitabine, navelbine, farnesyl-protein transferase inhibitors, transplatinum, 5-fluorouracil, vincristine, vinblastine and methotrexate, Temazolomide (an aqueous form of DTIC), or any analog or derivative variant of the foregoing. The combination of chemotherapy with biological therapy is known as biochemotherapy. The present invention contemplates any chemotherapeutic agent that may be employed or known in the art for treating or preventing cancers.

2. Radiotherapy

Other factors that cause DNA damage and have been used extensively include what are commonly known as γ -rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells. Other forms of DNA damaging factors are also contemplated such as microwaves and UV-irradiation. It is most likely that all of these factors effect a broad range of damage on DNA, on the precursors of DNA, on the replication and repair of DNA, and on the assembly and maintenance of chromosomes. Dosage ranges for X-rays range from daily doses of 50 to 200 roentgens for prolonged periods of time (3 to 4 wk), to single doses of 2000 to 6000 roentgens. Dosage ranges for radioisotopes vary widely, and depend on the half-life of the isotope, the strength and type of radiation emitted, and the uptake by the neoplastic cells.

The terms “contacted” and “exposed,” when applied to a cell, are used herein to describe the process by which a therapeutic construct and a chemotherapeutic or radiotherapeutic agent are delivered to a target cell or are placed in direct juxtaposition with the target cell. To achieve cell killing or stasis, both agents are delivered to a cell in a combined amount effective to kill the cell or prevent it from dividing.

3. Immunotherapy

Immunotherapeutics, generally, rely on the use of immune effector cells and molecules to target and destroy cancer cells. The immune effector may be, for example, an antibody specific for some marker on the surface of a tumor cell. The antibody alone may serve as an effector of therapy or it may recruit other cells to actually effect cell killing. The antibody also may be conjugated to a drug or toxin (chemotherapeutic, radionuclide, ricin A chain, cholera toxin, pertussis toxin, *etc.*) and serve merely as a targeting agent. Alternatively, the effector may be a lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a tumor cell target. Various effector cells include cytotoxic T-cells and NK cells. The combination of therapeutic modalities, *i.e.*, direct cytotoxic activity and inhibition or reduction of Fortilin would provide therapeutic benefit in the treatment of cancer.

Immunotherapy could also be used as part of a combined therapy. The general approach for combined therapy is discussed below. In one aspect of immunotherapy, the tumor cell must bear some marker that is amenable to targeting, *i.e.*, is not present on the majority of other cells. Many tumor markers exist and any of these may be suitable for targeting in the context of the present invention. Common tumor markers include carcinoembryonic antigen, prostate specific antigen, urinary tumor associated antigen, fetal antigen, tyrosinase (p97), gp68, TAG-72, HMFG, Sialyl Lewis Antigen, MucA, MucB, PLAP, estrogen receptor, laminin receptor, *erb B* and p155. An alternative aspect of immunotherapy is to anticancer effects with immune stimulatory effects. Immune stimulating molecules also exist including: cytokines such as IL-2, IL-4, IL-12, GM-CSF, gamma-IFN, chemokines such as MIP-1, MCP-1, IL-8 and growth factors such as FLT3 ligand. Combining immune stimulating molecules, either as proteins or using gene delivery in combination with a tumor suppressor such as mda-7 has been shown to enhance anti-tumor effects (Ju *et al.*, 2000).

As discussed earlier, examples of immunotherapies currently under investigation or in use are immune adjuvants (*e.g.*, *Mycobacterium bovis*, *Plasmodium falciparum*,

dinitrochlorobenzene and aromatic compounds) (U.S. Patent 5,801,005; U.S. Patent 5,739,169; Hui and Hashimoto, 1998; Christodoulides *et al.*, 1998), cytokine therapy (*e.g.*, interferons, and; IL-1, GM-CSF and TNF) (Bukowski *et al.*, 1998; Davidson *et al.*, 1998; Hellstrand *et al.*, 1998) gene therapy (*e.g.*, TNF, IL-1, IL-2, p53) (Qin *et al.*, 1998; Austin-Ward and Villaseca, 1998; U.S. Patent 5,830,880 and U.S. Patent 5,846,945) and monoclonal antibodies (*e.g.*, anti-ganglioside GM2, anti-HER-2, anti-p185) (Pietras *et al.*, 1998; Hanibuchi *et al.*, 1998; U.S. Patent 5,824,311). Herceptin (trastuzumab) is a chimeric (mouse-human) monoclonal antibody that blocks the HER2-neu receptor. It possesses anti-tumor activity and has been approved for use in the treatment of malignant tumors (Dillman, 1999). Combination therapy of cancer with herceptin and chemotherapy has been shown to be more effective than the individual therapies. Thus, it is contemplated that one or more anti-cancer therapies may be employed with the tumor-associated HLA-restricted peptide therapies described herein.

Adoptive Immunotherapy. In adoptive immunotherapy, the patient's circulating lymphocytes, or tumor infiltrated lymphocytes, are isolated *in vitro*, activated by lymphokines such as IL-2 or transduced with genes for tumor necrosis, and readministered (Rosenberg *et al.*, 1988; 1989). To achieve this, one would administer to an animal, or human patient, an immunologically effective amount of activated lymphocytes in combination with an adjuvant-incorporated antigenic peptide composition as described herein. The activated lymphocytes will most preferably be the patient's own cells that were earlier isolated from a blood or tumor sample and activated (or "expanded") *in vitro*. This form of immunotherapy has produced several cases of regression of melanoma and renal carcinoma, but the percentage of responders were few compared to those who did not respond.

Passive Immunotherapy. A number of different approaches for passive immunotherapy of cancer exist. They may be broadly categorized into the following: injection of antibodies alone; injection of antibodies coupled to toxins or chemotherapeutic agents; injection of antibodies coupled to radioactive isotopes; injection of anti-idiotypic antibodies; and finally, purging of tumor cells in bone marrow.

Preferably, human monoclonal antibodies are employed in passive immunotherapy, as they produce few or no side effects in the patient. However, their application is somewhat limited by their scarcity and have so far only been administered intralesionally. Human monoclonal antibodies to ganglioside antigens have been administered intralesionally to patients suffering from cutaneous recurrent melanoma (Irie & Morton, 1986). Regression was observed in six out of ten patients, following, daily or weekly, intralesional injections. In

another study, moderate success was achieved from intralesional injections of two human monoclonal antibodies (Irie *et al.*, 1989). Possible therapeutic antibodies include anti-TNF, anti-CD25, anti-CD3, anti-CD20, CTLA-4-IG, and anti-CD28.

It may be favorable to administer more than one monoclonal antibody directed against two different antigens or even antibodies with multiple antigen specificity. Treatment protocols also may include administration of lymphokines or other immune enhancers as described by Bajorin *et al.* (1988). The development of human monoclonal antibodies is described in further detail elsewhere in the specification.

4. Gene Therapy

In yet another embodiment, the secondary treatment is a gene therapy in which a therapeutic polynucleotide is administered before, after, or at the same time as the tumor-associated HLA-restricted peptide is administered. Delivery of a vector encoding a the tumor-associated HLA-restricted peptide in conjunction with a second vector encoding one of the following gene products will have a combined anti-hyperproliferative effect on target tissues. Alternatively, a single vector encoding both genes may be used. A variety of proteins are encompassed within the invention, some of which are described below. Various genes that may be targeted for gene therapy of some form in combination with the present invention are will known to one of ordinary skill in the art and may comprise any gene involved in cancers.

Inducers of Cellular Proliferation. The proteins that induce cellular proliferation further fall into various categories dependent on function. The commonality of all of these proteins is their ability to regulate cellular proliferation. For example, a form of PDGF, the sis oncogene, is a secreted growth factor. Oncogenes rarely arise from genes encoding growth factors, and at the present, sis is the only known naturally-occurring oncogenic growth factor. In one embodiment of the present invention, it is contemplated that anti-sense mRNA directed to a particular inducer of cellular proliferation is used to prevent expression of the inducer of cellular proliferation.

The proteins FMS, ErbA, ErbB and neu are growth factor receptors. Mutations to these receptors result in loss of regulatable function. For example, a point mutation affecting the transmembrane domain of the Neu receptor protein results in the neu oncogene. The erbA oncogene is derived from the intracellular receptor for thyroid hormone. The modified oncogenic ErbA receptor is believed to compete with the endogenous thyroid hormone receptor, causing uncontrolled growth.

The largest class of oncogenes includes the signal transducing proteins (*e.g.*, Src, Abl and Ras). The protein Src is a cytoplasmic protein-tyrosine kinase, and its transformation from proto-oncogene to oncogene in some cases, results *via* mutations at tyrosine residue 527. In contrast, transformation of GTPase protein ras from proto-oncogene to oncogene, in one example, results from a valine to glycine mutation at amino acid 12 in the sequence, reducing ras GTPase activity. The proteins Jun, Fos and Myc are proteins that directly exert their effects on nuclear functions as transcription factors.

Inhibitors of Cellular Proliferation. The tumor suppressor oncogenes function to inhibit excessive cellular proliferation. The inactivation of these genes destroys their inhibitory activity, resulting in unregulated proliferation. The most common tumor suppressors are Rb, p53, p21 and p16. Other genes that may be employed according to the present invention include APC, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, *zac1*, p73, VHL, C-CAM, MMAC1/PTEN, DBCCR-1, FCC, *rsk-3*, p27, p27/p16 fusions, and p21/p27 fusions.

Regulators of Programmed Cell Death. Apoptosis, or programmed cell death, is an essential process for normal embryonic development, maintaining homeostasis in adult tissues, and suppressing carcinogenesis (Kerr *et al.*, 1972). The Bcl-2 family of proteins and ICE-like proteases have been demonstrated to be important regulators and effectors of apoptosis in other systems. The Bcl-2 protein, discovered in association with follicular lymphoma, plays a prominent role in controlling apoptosis and enhancing cell survival in response to diverse apoptotic stimuli (Bakhshi *et al.*, 1985; Cleary and Sklar, 1985; Cleary *et al.*, 1986; Tsujimoto *et al.*, 1985; Tsujimoto and Croce, 1986). The evolutionarily conserved Bcl-2 protein now is recognized to be a member of a family of related proteins, which can be categorized as death agonists or death antagonists.

Subsequent to its discovery, it was shown that Bcl-2 acts to suppress cell death triggered by a variety of stimuli. Also, it now is apparent that there is a family of Bcl-2 cell death regulatory proteins that share in common structural and sequence homologies. These different family members have been shown to either possess similar functions to Bcl-2 (*e.g.*, Bcl_{XL}, Bcl_W, Bcl_S, Mcl-1, A1, Bfl-1) or counteract Bcl-2 function and promote cell death (*e.g.*, Bax, Bak, Bik, Bim, Bid, Bad, Harakiri).

5. Surgery

Approximately 60% of persons with cancer will undergo surgery of some type, which includes preventative, diagnostic or staging, curative and palliative surgery. Curative surgery is a cancer treatment that may be used in conjunction with other therapies, such as the

treatment of the present invention, chemotherapy, radiotherapy, hormonal therapy, gene therapy, immunotherapy and/or alternative therapies.

Curative surgery includes resection in which all or part of cancerous tissue is physically removed, excised, and/or destroyed. Tumor resection refers to physical removal of at least part of a tumor. In addition to tumor resection, treatment by surgery includes laser surgery, cryosurgery, electrosurgery, and microscopically controlled surgery (Mohs' surgery). It is further contemplated that the present invention may be used in conjunction with removal of superficial cancers, precancers, or incidental amounts of normal tissue.

Upon excision of part of all of cancerous cells, tissue, or tumor, a cavity may be formed in the body. Treatment may be accomplished by perfusion, direct injection or local application of the area with an additional anti-cancer therapy. Such treatment may be repeated, for example, every 1, 2, 3, 4, 5, 6, or 7 days, or every 1, 2, 3, 4, and 5 weeks or every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. These treatments may be of varying dosages as well.

C. Automimmune Diseases

The present invention also contemplates the treatment of autoimmune disease using the antibodies of the present invention. PR1 is derived from myeloid self-proteins. Proteinase 3 (Pr3), which contains PR1, is the target of autoimmune attack in Wegener's granulomatosis. Myeloperoxidase (MPO) is the target antigen in small vessel vasculitis (Franssen *et al.*, 1996; Brouwer *et al.*, 1994; Molldrem *et al.*, 1996), with evidence for both T-cell and antibody immunity in patients with these diseases. Wegener's granulomatosis is associated with production of cytoplasmic anti-neutrophil cytoplasmic antibodies (cANCA) with specificity for Pr3 (Molldrem *et al.*, 1997), while microscopic polyangiitis and Churg-Strauss syndrome are associated with perinuclear ANCA (pANCA) with specificity for MPO (Molldrem *et al.*, 1999; Savage *et al.*, 1999). As such, inhibiting immune cell recognition of PR1 may be therapeutic for autoimmune disease.

Thus, antibodies of the present invention will be administered to subjects suffering from autoimmune disease to neutralize effects of other autoantibodies (*e.g.*, pANCA against proteinase 3). Alternatively, an antibody will be engineered to be "bi-specific," *i.e.*, to have immunologic specificity for two antigen, where one is PR1/HLA-A2, and the other is a dendritic cell surface antigens like DEC-205, LOX-1, RAGE, thereby blocking dendritic cell function in antigen presentation.

1. Vasculitis

Vasculitis is a process caused by inflammation of blood vessel walls and results in a variety of disorders. An accepted classification system for vasculitis has not emerged, although it may be categorized by the size or type of the involved blood vessel as large-,
5 medium-, or small-vessel vasculitis. Small-vessel vasculitis is defined as vasculitis that affects vessels smaller than arteries (*i.e.*, arterioles, venules, and capillaries); however, small-vessel vasculitis can also involve medium-sized arteries. Anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis is the most common cause of small-vessel vasculitis and includes microscopic polyangiitis, Wegener's granulomatosis, Churg-Strauss syndrome,
10 and certain types of drug-induced vasculitis.

Wegener's Granulomatosis. Wegener's Granulomatosis is a rare disorder which causes inflammation of blood vessels in the upper respiratory tract (nose, sinuses, ears), lungs, and kidneys. Many other areas of the body may also be affected, with arthritis (joint inflammation) occurring in almost half of all cases. The eyes and skin may also be affected.
15 The cause is unknown, but Wegener's Granulomatosis is thought to be an autoimmune disorder and is often classified as one of the rheumatic diseases. Destructive lesions develop in the upper and lower respiratory tract and the kidney. In the kidney, these lesions cause glomerulonephritis that may result in hematuria (blood in the urine) and kidney failure. It occurs most often between the ages of 30 and 50, and men are affected twice as often as
20 women. It is rare in children, but has been seen in infants as young as 3 months old. The kidney disease can progress rapidly, with kidney failure occurring within months of the initial diagnosis. If untreated, kidney failure and death occur in more than 90% of all patients with Wegener's granulomatosis.

Early symptoms may include fatigue, malaise, fever, and a sense of discomfort around
25 the nose and sinuses. Upper respiratory infections such as sinusitis or ear infections frequently precede the diagnosis of Wegener's Granulomatosis. Other upper respiratory symptoms include nose bleeds, pain, and sores around the opening of the nose. Persistent fever without an obvious cause (fever of undetermined origin -- FUO) may be an initial symptom. Night sweats may accompany the fever. Loss of appetite and weight loss are
30 common. Skin lesions are common, but there is no one characteristic lesion associated with the disease. Kidney disease is necessary to make the definitive diagnosis of Wegener's Granulomatosis. The urine may be bloody, which often first appears as red or smoky urine. There may be no symptoms, but is easily diagnosed with laboratory studies. Eye problems

develop in a significant number of patients and may range from a mild conjunctivitis to severe inflammation of the eyeball and the tissues around the eyeball. Additional symptoms include weakness, loss of appetite, weight loss, bloody discharge from the nose, pain over the sinuses, sinusitis, lesions in and around the opening of the nose, cough, coughing up blood, bloody sputum, shortness of breath, wheezing, chest pain, blood in the urine, rashes, and joint pain.

Diagnosis is made by taking a biopsy of abnormal tissue, which may include open lung biopsy, upper airway biopsy, nasal mucosal biopsy, bronchoscopy with transtracheal biopsy, kidney biopsy, urinalysis, chest x-ray, bone marrow aspiration, blood test (for autoantibodies). Treatment includes corticosteroids, cyclophosphamide, methotrexate, or azathioprine, which can produce long-term remission in over 90% of affected people.

Churg-Strauss Syndrome. Churg-Strauss Syndrome (CSS), also known as allergic granulomatosis, is a form of systemic vasculitis. CSS is similar to polyarteritis nodosa, but the abundance of eosinophils distinguished this disease. Most CSS patients are middle-aged, with a history of new or increased severity asthma - asthma being one of the cardinal features of CSS. The symptoms of asthma may begin long before the onset of vasculitis. Other early symptoms include nasal polyps and allergic rhinitis. The disease often transitions into eosinophilia, with counts reaching as high as 60%. The next phase of disease is overt vasculitis, which can involve the skin, lungs, nerves, kidneys, and other organs. Peripheral nerve involvement can be particularly debilitating and includes pain, numbness, or tingling in extremities (neuropathy/mononeuritis multiplex). Prior to the advent of therapies, CSS was often a fatal disease. The majority of patients died from rampant, uncontrolled disease.

The cause of CSS is not known, but it is likely multi-factorial. Though a genetic factor may exist, CSS is only rarely seen in two members of the same family. Thus, environmental factors and infections are more likely to be the cause, but there is no definitive evidence of this. Diagnosis is performed by a specific combination of symptoms and signs, the pattern of organ involvement, and the presence of certain abnormal blood tests (eosinophilia, in particular). In addition to a detailed patient history and physical examination, blood tests, chest X-rays and other types of imaging studies, nerve conduction tests, and tissue biopsies (lung, skin, or nerve) may be performed to aid in the diagnosis. In order to be classified as a CSS patient, a patient should have at least 4 of the following 6 criteria: 1) asthma; 2) eosinophilia [$>10\%$ on differential WBC count]; 3) mononeuropathy; 4) transient pulmonary infiltrates on chest X-rays; 5) paranasal sinus abnormalities; and 6) biopsy containing a blood vessel with extravascular eosinophils.

CSS usually responds to prednisone. Initially, high doses of oral prednisone are used, but after the first month or so, this high dose of prednisone is gradually tapered down over the ensuing months. Other immunosuppressive drugs, such as azathioprine, cellcept, methotrexate, or cyclophosphamide may be used in addition to prednisone. High doses of intravenous steroids may be useful for those patients with severe disease, or for those who are unresponsive to other treatments. With proper therapy, symptoms begin to resolve quickly, with gradual improvement in cardiac and renal function, as well as improvement in the pain that results from peripheral nerve involvement. Therapy may last for 1 to 2 years, depending on patient response and continuation of disease.

2. Crohn's Disease

Crohn's disease symptoms include intestinal inflammation and the development of intestinal stenosis and fistulas; neuropathy often accompanies these symptoms. Anti-inflammatory drugs, such as 5-aminosalicylates (*e.g.*, mesalamine) or corticosteroids, are typically prescribed, but are not always effective (reviewed in V. A. Botoman *et al.*, 1998). Immunosuppression with cyclosporine is sometimes beneficial for patients resistant to or intolerant of corticosteroids (Brynskov *et al.*, 1989).

Nevertheless, surgical correction is eventually required in 90% of patients; 50% undergo colonic resection (Leiper *et al.*, 1998; Makowiec *et al.*, 1998). The recurrence rate after surgery is high, with 50% requiring further surgery within 5 years (Leiper *et al.*, 1998; Besnard *et al.*, 1998).

One hypothesis for the etiology of Crohn's disease is that a failure of the intestinal mucosal barrier, possibly resulting from genetic susceptibilities and environmental factors (*e.g.*, smoking), exposes the immune system to antigens from the intestinal lumen including bacterial and food antigens (*e.g.*, Soderholm *et al.*, 1999; Hollander *et al.*, 1986; Hollander, 1992). Another hypothesis is that persistent intestinal infection by pathogens such as *Mycobacterium paratuberculosis*, *Listeria monocytogenes*, abnormal *Escherichia coli*, or paramyxovirus, stimulates the immune response; or alternatively, symptoms result from a dysregulated immune response to ubiquitous antigens, such as normal intestinal microflora and the metabolites and toxins they produce (Sartor, 1997). The presence of IgA and IgG anti-*Saccharomyces cerevisiae* antibodies (ASCA) in the serum was found to be highly diagnostic of pediatric Crohn's disease (Ruemmele *et al.*, 1998; Hoffenberg *et al.*, 1999).

In Crohn's disease, a dysregulated immune response is skewed toward cell-mediated immunopathology (Murch, 1998). But immunosuppressive drugs, such as cyclosporine, tacrolimus, and mesalamine have been used to treat corticosteroid-resistant cases of Crohn's disease with mixed success (Brynskov *et al.*, 1989; Fellerman *et al.*, 1998).

5 Recent efforts to develop diagnostic and treatment tools against Crohn's disease have focused on the central role of cytokines (Schreiber, 1998; van Hogezaand & Verspaget, 1998). Cytokines are small, secreted proteins or factors (5 to 20 kD) that have specific effects on cell-to-cell interactions, intercellular communication, or the behavior of other cells. Cytokines are produced by lymphocytes, especially T_H1 and T_H2 lymphocytes, monocytes, intestinal
10 macrophages, granulocytes, epithelial cells, and fibroblasts (reviewed in Rogler & Andus, 1998; Galley & Webster, 1996). Some cytokines are pro-inflammatory (*e.g.*, TNF- α , IL-1(α and β), IL-6, IL-8, IL-12, or leukemia inhibitory factor (LIF)); others are anti-inflammatory (*e.g.*, IL-1 receptor antagonist, IL-4, IL-10, IL-11, and TGF- β). However, there may be overlap and functional redundancy in their effects under certain inflammatory conditions.

15 In active cases of Crohn's disease, elevated concentrations of TNF- α and IL-6 are secreted into the blood circulation, and TNF- α , IL-1, IL-6, and IL-8 are produced in excess locally by mucosal cells (Funakoshi *et al.*, 1998). These cytokines can have far-ranging effects on physiological systems including bone development, hematopoiesis, and liver, thyroid, and neuropsychiatric function. Also, an imbalance of the IL-1 β /IL-1ra ratio, in favor
20 of pro-inflammatory IL-1 β , has been observed in patients with Crohn's disease (Rogler & Andus, 1998; Saiki *et al.*, 1998; Dionne *et al.*, 1998; but see S. Kuboyama, 1998). One study suggested that cytokine profiles in stool samples could be a useful diagnostic tool for Crohn's disease (Saiki *et al.*, 1998).

Treatments that have been proposed for Crohn's disease include the use of various
25 cytokine antagonists (*e.g.*, IL-1ra), inhibitors (*e.g.*, of IL-1 β converting enzyme and antioxidants) and anti-cytokine antibodies (Rogler and Andus, 1998; van Hogezaand & Verspaget, 1998; Reimund *et al.*, 1998; N. Lugerling *et al.*, 1998; McAlindon *et al.*, 1998). In particular, monoclonal antibodies against TNF- α have been tried with some success in the treatment of Crohn's disease (Targan *et al.*, 1997; Stack *et al.*, 1997; van Dullemen *et al.*,
30 1995). These compounds can be used in combination therapy with compounds of the present invention.

Another approach to the treatment of Crohn's disease has focused on at least partially eradicating the bacterial community that may be triggering the inflammatory response and

replacing it with a non-pathogenic community. For example, U.S. Patent 5,599,795 discloses a method for the prevention and treatment of Crohn's disease in human patients. Their method was directed to sterilizing the intestinal tract with at least one antibiotic and at least one anti-fungal agent to kill off the existing flora and replacing them with different, select, well-
5 characterized bacteria taken from normal humans. Borody taught a method of treating Crohn's disease by at least partial removal of the existing intestinal microflora by lavage and replacement with a new bacterial community introduced by fecal inoculum from a disease-screened human donor or by a composition comprising *Bacteroides* and *Escherichia coli* species (U.S. Patent 5,443,826). However, there has been no known cause of Crohn's disease
10 to which diagnosis and/or treatment could be directed.

3. Rheumatoid Arthritis

The exact etiology of RA remains unknown, but it is clear that it has autoimmune aspects. The first signs of joint disease appear in the synovial lining layer, with proliferation of synovial fibroblasts and their attachment to the articular surface at the joint margin
15 (Lipsky, 1998). Subsequently, macrophages, T-cells and other inflammatory cells are recruited into the joint, where they produce a number of mediators, including the cytokines interleukin-1 (IL-1), which contributes to the chronic sequelae leading to bone and cartilage destruction, and tumour necrosis factor (TNF- α), which plays a role in inflammation (Dinarello, 1998; Arend & Dayer, 1995; van den Berg, 2001). The concentration of IL-1 in
20 plasma is significantly higher in patients with RA than in healthy individuals and, notably, plasma IL-1 levels correlate with RA disease activity (Eastgate *et al.*, 1988). Moreover, synovial fluid levels of IL-1 are correlated with various radiographic and histologic features of RA (Kahle *et al.*, 1992; Rooney *et al.*, 1990).

In normal joints, the effects of these and other proinflammatory cytokines are
25 balanced by a variety of anti-inflammatory cytokines and regulatory factors (Burger & Dayer, 1995). The significance of this cytokine balance is illustrated in juvenile RA patients, who have cyclical increases in fever throughout the day (Prieur *et al.*, 1987). After each peak in fever, a factor that blocks the effects of IL-1 is found in serum and urine. This factor has been isolated, cloned and identified as IL-1 receptor antagonist (IL-1ra), a member of the IL-1 gene
30 family (Hannum *et al.*, 1990). IL-1ra, as its name indicates, is a natural receptor antagonist that competes with IL-1 for binding to type I IL-1 receptors and, as a result, blocks the effects of IL-1 (Arend *et al.*, 1998). A 10- to 100-fold excess of IL-1ra may be needed to block IL-1

effectively; however, synovial cells isolated from patients with RA do not appear to produce enough IL-1ra to counteract the effects of IL-1 (Firestein *et al.*, 1994; Fujikawa *et al.*, 1995).

4. Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune rheumatic disease characterized by deposition in tissues of autoantibodies and immune complexes leading to tissue injury (Kotzin, 1996). In contrast to autoimmune diseases such as MS and type 1 diabetes mellitus, SLE potentially involves multiple organ systems directly, and its clinical manifestations are diverse and variable (reviewed by Kotzin & O'Dell, 1995). For example, some patients may demonstrate primarily skin rash and joint pain, show spontaneous remissions, and require little medication. At the other end of the spectrum are patients who demonstrate severe and progressive kidney involvement that requires therapy with high doses of steroids and cytotoxic drugs such as cyclophosphamide (Kotzin, 1996).

The serological hallmark of SLE, and the primary diagnostic test available, is elevated serum levels of IgG antibodies to constituents of the cell nucleus, such as double-stranded DNA (dsDNA), single-stranded DNA (ss-DNA), and chromatin. Among these autoantibodies, IgG anti-dsDNA antibodies play a major role in the development of lupus glomerulonephritis (GN) (Hahn & Tsao, 1993; Ohnishi *et al.*, 1994). Glomerulonephritis is a serious condition in which the capillary walls of the kidney's blood purifying glomeruli become thickened by accretions on the epithelial side of glomerular basement membranes. The disease is often chronic and progressive and may lead to eventual renal failure.

The mechanisms by which autoantibodies are induced in these autoimmune diseases remain unclear. As there has been no known cause of SLE, to which diagnosis and/or treatment could be directed, treatment has been directed to suppressing immune responses, for example with macrolide antibiotics, rather than to an underlying cause. (*e.g.*, U.S. Patent 4,843,092).

5. Juvenile Rheumatoid Arthritis

Juvenile rheumatoid arthritis (JRA), a term for the most prevalent form of arthritis in children, is applied to a family of illnesses characterized by chronic inflammation and hypertrophy of the synovial membranes. The term overlaps, but is not completely synonymous, with the family of illnesses referred to as juvenile chronic arthritis and/or juvenile idiopathic arthritis in Europe.

Jarvis (1998) and others (Arend, 2001) have proposed that the pathogenesis of rheumatoid disease in adults and children involves complex interactions between innate and adaptive immunity. This complexity lies at the core of the difficulty of unraveling disease pathogenesis.

Both innate and adaptive immune systems use multiple cell types, a vast array of cell surface and secreted proteins, and interconnected networks of positive and negative feedback (Lo *et al.*, 1999). Furthermore, while separable in thought, the innate and adaptive wings of the immune system are functionally intersected (Fearon & Locksley, 1996), and pathologic events occurring at these intersecting points are likely to be highly relevant to our understanding of pathogenesis of adult and childhood forms of chronic arthritis (Warrington, *et al.*, 2001).

Polyarticular JRA is a distinct clinical subtype characterized by inflammation and synovial proliferation in multiple joints (four or more), including the small joints of the hands (Jarvis, 2002). This subtype of JRA may be severe, because of both its multiple joint involvement and its capacity to progress rapidly over time. Although clinically distinct, polyarticular JRA is not homogeneous, and patients vary in disease manifestations, age of onset, prognosis, and therapeutic response. These differences very likely reflect a spectrum of variation in the nature of the immune and inflammatory attack that can occur in this disease (Jarvis, 1998).

6. Sjögren's syndrome

Primary Sjögren's syndrome (SS) is a chronic, slowly progressive, systemic autoimmune disease, which affects predominantly middle-aged women (female-to-male ratio 9:1), although it can be seen in all ages including childhood (Jonsson *et al.*, 2002). It is characterized by lymphocytic infiltration and destruction of the exocrine glands, which are infiltrated by mononuclear cells including CD4+, CD8+ lymphocytes and B-cells (Jonsson *et al.*, 2002). In addition, extraglandular (systemic) manifestations are seen in one-third of patients (Jonsson *et al.*, 2001).

The glandular lymphocytic infiltration is a progressive feature (Jonsson *et al.*, 1993), which, when extensive, may replace large portions of the organs. Interestingly, the glandular infiltrates in some patients closely resemble ectopic lymphoid microstructures in the salivary glands (denoted as ectopic germinal centers) (Salomonsson *et al.*, 2002; Xanthou & Polihronis, 2001). In SS, ectopic GCs are defined as T and B cell aggregates of proliferating cells with a network of follicular dendritic cells and activated endothelial cells. These GC-like

structures formed within the *target* tissue also portray functional properties with production of autoantibodies (anti-Ro/SSA and anti-La/SSB) (Salomonsson &, Jonsson, 2003).

In other systemic autoimmune diseases, such as RA, factors critical for ectopic GCs have been identified. Rheumatoid synovial tissues with GCs were shown to produce chemokines CXCL13, CCL21 and lymphotoxin (LT)- β (detected on follicular center and mantle zone B cells). Multivariate regression analysis of these analytes identified CXCL13 and LT- β as the solitary cytokines predicting GCs in rheumatoid synovitis (Weyand & Goronzy, 2003). Recently CXCL13 and CXCR5 in salivary glands has been shown to play an essential role in the inflammatory process by recruiting B and T-cells, therefore contributing to lymphoid neogenesis and ectopic GC formation in SS (Salomonsson & Larsson, 2002).

7. Psoriasis

Psoriasis is a chronic skin disease of scaling and inflammation that affects 2 to 2.6 percent of the United States population, or between 5.8 and 7.5 million people. Although the disease occurs in all age groups, it primarily affects adults. It appears about equally in males and females. Psoriasis occurs when skin cells quickly rise from their origin below the surface of the skin and pile up on the surface before they have a chance to mature. Usually this movement (also called turnover) takes about a month, but in psoriasis it may occur in only a few days. In its typical form, psoriasis results in patches of thick, red (inflamed) skin covered with silvery scales. These patches, which are sometimes referred to as plaques, usually itch or feel sore. They most often occur on the elbows, knees, other parts of the legs, scalp, lower back, face, palms, and soles of the feet, but they can occur on skin anywhere on the body. The disease may also affect the fingernails, the toenails, and the soft tissues of the genitals and inside the mouth. While it is not unusual for the skin around affected joints to crack, approximately 1 million people with psoriasis experience joint inflammation that produces symptoms of arthritis. This condition is called psoriatic arthritis.

Psoriasis is a skin disorder driven by the immune system, especially involving T-cells. In psoriasis, T-cells are put into action by mistake and become so active that they trigger other immune responses, which lead to inflammation and to rapid turnover of skin cells. In about one-third of the cases, there is a family history of psoriasis. Researchers have studied a large number of families affected by psoriasis and identified genes linked to the disease. People with psoriasis may notice that there are times when their skin worsens, then improves. Conditions that may cause flareups include infections, stress, and changes in climate that dry

the skin. Also, certain medicines, including lithium and betablockers, which are prescribed for high blood pressure, may trigger an outbreak or worsen the disease.

8. Multiple Sclerosis

Multiple sclerosis (MS) continues to be a serious health problem that afflicts hundreds of thousands each year in the US alone, and millions worldwide. It is one of the most common diseases of the central nervous system (brain and spinal cord). MS is an inflammatory condition associated with demyelination, or loss of the myelin sheath. Myelin, a fatty material that insulates nerves, acts as insulator in allowing nerves to transmit impulses from one point to another. In MS, the loss of myelin is accompanied by a disruption in the ability of the nerves to conduct electrical impulses to and from the brain and this produces the various symptoms of MS, such as impairments in vision, muscle coordination, strength, sensation, speech and swallowing, bladder control, sexuality and cognitive function. The plaques or lesions where myelin is lost appear as hardened, scar-like areas. These scars appear at different times and in different areas of the brain and spinal cord, hence the term “multiple” sclerosis, literally meaning many scars.

Currently, there is no single laboratory test, symptom, or physical finding that provides a conclusive diagnosis of MS. To complicate matters, symptoms of MS can easily be confused with a wide variety of other diseases such as acute disseminated encephalomyelitis, Lyme disease, HIV-associated myelopathy, HTLV-I-associated myelopathy, neurosyphilis, progressive multifocal leukoencephalopathy, systemic lupus erythematosus, polyarteritis nodosa, Sjögren's syndrome, Behçet's disease, sarcoidosis, paraneoplastic syndromes, subacute combined degeneration of cord, subacute myelo-optic neuropathy, adrenomyeloneuropathy, spinocerebellar syndromes, hereditary spastic paraparesis/primary lateral sclerosis, strokes, tumors, arteriovenous malformations, arachnoid cysts, Arnold-Chiari malformations, and cervical spondylosis. Consequently, the diagnosis of MS must be made by a process that demonstrates findings consistent with MS, and also rules out other causes.

Generally, the diagnosis of MS relies on two criteria. First, there must have been two attacks at least one month apart. An attack, also known as an exacerbation, flare, or relapse, is a sudden appearance of or worsening of an MS symptom or symptoms which lasts at least 24 hours. Second, there must be more than one area of damage to central nervous system myelin sheath. Damage to sheath must have occurred at more than one point in time and not have been caused by any other disease that can cause demyelination or similar neurologic

symptoms. MRI (magnetic resonance imaging) currently is the preferred method of imaging the brain to detect the presence of plaques or scarring caused by MS.

The diagnosis of MS cannot be made, however, solely on the basis of MRI. Other diseases can cause comparable lesions in the brain that resemble those caused by MS. Furthermore, the appearance of brain lesions by MRI can be quite heterogeneous in different patients, even resembling brain or spinal cord tumors in some. In addition, a normal MRI scan does not rule out a diagnosis of MS, as a small number of patients with confirmed MS do not show any lesions in the brain on MRI. These individuals often have spinal cord lesions or lesions which cannot be detected by MRI. As a result, it is critical that a thorough clinical exam also include a patient history and functional testing. This should cover mental, emotional, and language functions, movement and coordination, vision, balance, and the functions of the five senses. Sex, birthplace, family history, and age of the person when symptoms first began are also important considerations. Other tests, including evoked potentials (electrical diagnostic studies that may reveal delays in central nervous system conduction times), cerebrospinal fluid (seeking the presence of clonally-expanded immunoglobulin genes, referred to as oligoclonal bands), and blood (to rule out other causes), may be required in certain cases.

D. Combination Therapy

Combination therapies for the immune disorders listed above is also contemplated. Such therapies would include standard therapies such as anti-inflammatories and immunosuppressive agents, used in conjunction with the therapeutic methods of the present invention. Such standard therapies would be capable of negatively affecting an immune cell causing disease in a subject or to alleviate the symptoms of such disease. This process may involve contacting the cells or subject with the both agent(s) at the same time. This may be achieved with a single composition or pharmacological formulation that includes both agents, or with two distinct compositions or formulations at the same time. Alternatively, the antibody therapy may precede or follow the other agent treatment by intervals ranging from minutes to weeks.

Various combinations may be employed; for example, the antibody therapy (with or without a conjugated therapeutic agent) is “A” and the secondary immune disease therapy is “B”:

A/B/A	B/A/B	B/B/A	A/A/B	A/B/B	B/A/A	A/B/B/B	B/A/B/B
B/B/B/A	B/B/A/B	A/A/B/B	A/B/A/B	A/B/B/A	B/B/A/A		
B/A/B/A	B/A/A/B	A/A/A/B	B/A/A/A	A/B/A/A	A/A/B/A		

5 Administration of the therapeutic agents of the present invention to a patient will follow general protocols for the administration of that particular secondary therapy, taking into account the toxicity, if any, of the antibody therapy. It is expected that the treatment cycles would be repeated as necessary. It also is contemplated that various standard therapies, as well as surgical intervention, may be applied in combination with the described therapies.

10 VII. EXAMPLES

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for
15 its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

20 EXAMPLE 1: METHODS

Antibody production. To obtain an antibody against the combined PR1/HLA-A*0201 epitope, the inventors immunized BALB/c mice with recombinant PR1/HLA-A*0201 monomers via subcutaneous (SQ) and intraperitoneal (IP) routes, three times spaced
25 two weeks apart. Splenocytes were isolated from the immunized animal and B cells were fused with HGPRT negative, immortalized myeloma cells using polyethylene glycol (PEG). Hybridoma cells were then selected with pp65/HLA-A*0201 and PR1/HLA-A*0201 monomers and placed into 96-well plates for single cell cloning.

Antibody Screening and Characterization. Monoclonal cell lines (~20,000) were
30 screened with PR1/HLA-A*0201 monomers by ELISA to identify a positive antibody-secreting hybridoma. The 8F4 hybridoma was identified by ELISA with specificity for PR1/HLA-A*0201 and was characterized using isotype-specific antibodies and immunoglobulin light chain antibodies.

Antibody Cloning, Sequence Analysis and Binding Studies. 8F4 heavy chain was cloned from hybridoma cDNA and primary sequence was obtained. Epitope mapping was performed by folding altered PR1 peptides, containing an Ala substitution at each P1 to P9 position, with the HLA-A*0201 heavy chain plus β -2 micro globulin. Binding affinity of 8F4 to PR1/HLA-A*0201 was determined by surface Plasmon resonance on a Biacore instrument with immobilized 8F4 and increasing concentration of soluble PR1/HLA-A*0201. FACS analysis and confocal imaging were utilized to study the binding of 8F4 to normal and abnormal cells.

Antibody Activity. To determine whether binding of 8F4 to AML triggers cell lysis, antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) assays were performed. AML cells from patient material that was shown to be sensitive to 8F4 CDC-mediated lysis were incubated in the presence or absence of 8F4 or isotype control and then transferred into irradiated (200 cGy) immunodeficient HLA-A2 transgenic NOD/SCID mice. At two weeks the animals were sacrificed and splenocytes and bone marrow were analyzed by FACS.

Total RNA isolation. Qiagen RNA easy kit with minelut columns were used. RNA loading buffer was made as follows: add 1 μ l ethidium bromide (EtBr) (10 mg/ml) to 100 μ l, 10x DNA loading dye, 1% agarose in 1X TAE, RNase free H₂O (in kit). Instructions are "Frozen vial of hybridoma cells or 1-5 x 10⁶ live cells. If active cultures are available, pellet 1-5 x 10⁶ live cells in 15 ml conical tube as in step 4 and proceed to step 5. If only frozen cells are available, thaw 1 vial hybridoma cells at 37C, remove from water bath promptly after thaw, mix gently. Wipe vial with 70% Ethanol and unscrew cap taking care to avoid touching threads. Transfer content of vial to 15 ml conical tube containing 15 ml complete media. Centrifuge 100 x g (~1,000 rpm for low speed Sorvall centrifuge) for 5 min. During spin, add b-mercaptoethanol to small aliquot of RLT buffer. Carefully remove all the media from cells with 10 ml pipette. Lyse cell pellet in buffer RLT using Qias shredder and follow Qiagen protocol for RNA isolation. Elute RNA from minelut column with 2X of 15 μ l RNase free dH₂O depending on starting cell amount (if from 6-well, elute with 1 x 13 μ l). RNA should remain on ICE throughout following procedures. Pour 1% agarose minigel containing 1 μ g/ml EtBr and during 15 min solidification time quantitate RNA. Quantitate 2 μ l RNA using spectrophotometer, using same RNase free H₂O above as a blank. Calculate RNA concentration: (A₂₆₀)(40) – μ g/ml. A260/280 ratio should be >1.6. Check quality of RNA by running 1 μ g on 1% agarose minigel in 10 μ l total volume of 1X RNA loading buffer. Run ~1

inch into the gel. Analyze gel on photo documentation system. The banding pattern of high quality RNA is characterized by distinct 28s and 18s ribosomal RNA bands at an ideal ratio of 2:1 in intensity. A 1:1 ratio may be acceptable, however no bands or a smear at the bottom of the gel is indicative of RNA degradation and indicates that this RNA should not be used.”

5 **Isolation and sequence analysis of rearranged Ig variable region (V) genes from hybridoma.** To obtain DNA sequences from V heavy chain (VH) and V light chain (VL) genes, the rapid amplification of cDNA ends (RACE) PCR in combination with human heavy chain constant region primers or light chain constant region primers was used. 5'RACE cDNA amplification was performed with the BD Smart TM RACE cDNA amplification kit
10 (BD Bioscience) and followed the instructions provided therewith. PFU ultra (Stratagene), Universla primer A mix (UPM) and gene specific primers (GSP) for human IgG H&L constant region were utilized.

Cloning and DNA sequencing of 5' RACE PCR products used the TOPO cloning kit (Invitrogen) and gel extraction kit (Qiagen). For IgG L, 8 colonies were islated for miniprep
15 and screen by EcoRI digest. Six positive clones were sequenced with M13 rev and T7 primers. For IgG, 8 colonies were isolated for miniprep and screen by EcoRI digest. Six positive clones were sequenced with M13 rev and T7 primers.

EXAMPLE 2: RESULTS

20 **Antibody production.** To obtain an antibody against the combined PR1/HLA-A*0201 epitope, the inventors immunized BALB/c mice with recombinant PR1/HLA-A*0201 monomers via subcutaneous (SQ) and intraperitoneal (IP) routes, three times spaced two weeks apart. Splenocytes were isolated from the immunized animal and B cells were
25 fused with HGPRT negative, immortalized myeloma cells using polyethylene glycol (PEG). Hybridoma cells were then selected with pp65/HLA-A*0201 and PR1/HLA-A*0201 monomers and placed into 96-well plates for single cell cloning.

30 **Antibody Screening and Characterization.** Monoclonal cell lines were screened with PR1/HLA-A*0201 monomers by ELISA to identify a positive antibody-secreting hybridoma. Nearly 2,000 hybridomas were screened and one, dubbed 8F4, was identified by ELISA with specificity for PR1/HLA-A*0201. The 8F4 hybridoma was characterized using isotype-specific antibodies and immunoglobulin light chain antibodies and shown to secrete a single IgG2a-κ PR1/HLA-A*0201-specific antibody.

Antibody Cloning, Sequence Analysis and Binding Assessment. 8F4 heavy chain was cloned from hybridoma cDNA and primary sequence was obtained. Epitope mapping was performed by folding altered PR1 peptides, containing an Ala substitution at each P1 to P9 position, with the HLA-A*0201 heavy chain plus $\beta 2$ microglobulin. P1 turned out to be most critical for 8F4 binding, although alteration of all amino acid positions disrupted binding (FIG. 1). Binding affinity of 8F4 to PR1/HLA-A*0201 was determined by surface Plasmon resonance on a Biacore instrument with immobilized 8F4 and increasing concentration of soluble PR1/HLA-A*0201, as shown in FIG. 2. 8F4 K_D is 9.9 nM, compared to K_D of 162 nM for a commercially available BB7.2 murine monoclonal antibody that recognizes a distinct allele-specific site on HLA-A*0201. Using confocal microscopy, direct fluorescence conjugates of 8F4 only bound to PR1 peptide-pulsed T2 cells (that express HLA-A*0201), but not to irrelevant pp65-pulsed or to non-pulsed T2 cells. Taken together, 8F4 specificity for, and high 8F4 binding affinity to the combined PR1/HLA-A*0201 was confirmed. Using both FACS analysis and confocal imaging (again with 8F4, FITC-conjugated BB7.2 anti-HLA-A*0201 antibody, and DAPI), 8F4 was shown to bind to circulating blasts from HLA-A2+ patients with AML but not to PBMC from HLA-A2+ healthy donors nor to HLA-A2 negative AML blasts (FIGS. 3 and 5).

Antibody Action Against Target Cells. To determine whether binding of 8F4 to AML triggers cell lysis, antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) assays were performed. CDC-mediated lysis of HLA-A2+ AML by 8F4, but not HLA-A2 negative AML or HLA-A2+ healthy donor control PBMC, was shown to be antibody dose-dependent (FIG. 4). AML cells from patient material that was shown to be sensitive to 8F4 CDC-mediated lysis were incubated in the presence or absence of 8F4 or isotype control and then transferred into irradiated (200 cGy) immunodeficient HLA-A2 transgenic NOD/SCID mice. At two weeks the animals were sacrificed and splenocytes and bone marrow were analyzed by FACS. At necropsy, AML was identified only in the IgG2a isotype control-treated animal but not in the 8F4-treated animal (FIG. 6). There was no apparent toxicity in the mice that received 8F4 alone compared to the isotype-treated mice. In total, these data support the conclusion that 8F4 monoclonal antibody: (1) specifically binds with high affinity to the combined PR1/HLA-A*0201 epitope; (2) specifically binds to, and can be used to identify PR1 peptide-occupied HLA-A*0201 molecules on the surface of human cells, including myeloid leukemia; (3) causes specific

lysis of HLA-A2+ AML in the presence of complement; (4) can prevent engraftment of AML in an immunodeficient mouse model.

Prevention of Tumor Engraftment. AML infiltration in tissues of experimental mice following injection with AML cells plus 8F4 was measured and is shown in FIGS. 8A-B. AML cells were not detected in the bone marrow and peripheral blood of no transfer control and experimental 8F4-treated mice. Mice that received AML cells mixed with isotype matched control antibody (iso) showed engraftment of AML1 and AML5 two or four weeks after AML transfer. An extended panel, including a mouse cell specific marker (mCD45), 3-6 human markers (CD45, CD13, CD33, CD34, CD38, HLA-DR), and Live/Dead Fixable Aqua (Invitrogen) was used for flow cytometric analysis of AML engraftment. All plots show viable mCD45- cells.

8F4 Induces Transient Neutropenia in HLA-A2 Transgenic NOD/SCID. HLA-A2 Tg NOD/SCID, shown to present endogenous PR1, were injected with 8F4 or control Ab. Bone marrow cells were harvested and stained with mAb directed to mouse antigens. Reduced granulocytes were evident in scatter profiles of bone marrow (FIG. 9A; left panels). Gr-1lo immature neutrophils were present, but Gr-1hi mature neutrophils were less numerous in the bone marrow of 8F4-treated mice (FIG. 9A; center panels). Additionally, monocytes (SSC^{lo} CD11b⁺; FIG. 9A; lower right gate of right panels) were reduced in 8F4-treated animals. Intravenous injection of 8F4 induced transient reduction in absolute numbers of circulating mature granulocytes, macrophages and monocytes in HLA-A2 Tg NOD/SCID mice (FIG. 9B). Three weeks after treatment, all populations remain. No significant pathological changes were evident in liver, lung, spleen, kidney, heart or brains of HLA-A2 Tg NOD/SCID mice 7 days after injection of 200 µg (10 mg/kg) 8F4 (FIG. 9C).

8F4 Induces Transient Leukopenia of Established Human Hematopoietic Cells. Peripheral blood from mice was taken to monitor cord blood engraftment, and 9-12 weeks after transfer mice were injected with 8F4. Mice were subsequently sacrificed and blood, spleen and bone marrow were analyzed for engraftment of human cells (FIG. 10B). As can be seen, the antibody injection transiently reduces the % engraftment of transferred cells (FIG. 10A).

* * * * *

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred

embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and
5 physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

VIII. REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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- U.S. Patent 4,472,509
- U.S. Patent 4,554,101
- U.S. Patent 4,680,338
- U.S. Patent 4,684,611
- 10 U.S. Patent 4,797,368
- U.S. Patent 4,843,092
- U.S. Patent 4,879,236
- U.S. Patent 4,938,948
- U.S. Patent 4,952,500
- 15 U.S. Patent 5,021,236
- U.S. Patent 5,139,648
- U.S. Patent 5,141,941
- U.S. Patent 5,302,523
- U.S. Patent 5,322,783
- 20 U.S. Patent 5,384,253
- U.S. Patent 5,399,363
- U.S. Patent 5,443,826
- U.S. Patent 5,464,765
- U.S. Patent 5,466,468
- 25 U.S. Patent 5,538,877
- U.S. Patent 5,538,880
- U.S. Patent 5,543,158
- U.S. Patent 5,550,318
- U.S. Patent 5,563,055
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- U.S. Patent 5,580,859
- U.S. Patent 5,589,466
- U.S. Patent 5,599,795
- U.S. Patent 5,610,042

- U.S. Patent 5,641,515
U.S. Patent 5,656,610
U.S. Patent 5,702,932
U.S. Patent 5,736,524
5 U.S. Patent 5,739,169
U.S. Patent 5,780,448
U.S. Patent 5,789,215
U.S. Patent 5,801,005
U.S. Patent 5,824,311
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U.S. Patent 5,846,233
U.S. Patent 5,846,945
U.S. Patent 5,856,456
U.S. Patent 5,871,986
15 U.S. Patent 5,880,270
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CLAIMS

1. An isolated and purified antibody that binds to VLQELNVTV (SEQ ID NO:1) when bound by an HLA-A2 receptor.
5
2. The antibody of claim 1, wherein (a) the heavy chain variable region CDR1-3 segments comprise SEQ ID NOS:3, 5 and 7; or (b) wherein the light chain variable region segments comprising SEQ ID NOS:15, 19 and 23; or (c) both (a) and (b)
- 10 2. The antibody of claim 2, wherein the heavy chain variable region sequence comprises SEQ ID NO:9 or 25, and/or the light chain variable region sequence comprises SEQ ID NO:24.
3. The antibody of claim 1, wherein said antibody is a single chain antibody.
15
4. The antibody of claim 1, wherein said antibody is fused to a non-antibody peptide or polypeptide segment.
5. The antibody of claim 1, wherein said antibody is linked to a diagnostic reagent.
20
6. The antibody of claim 5, wherein said diagnostic reagent is a fluorophore, a chromophore, a dye, a radioisotope, a chemiluminescent molecule, a paramagnetic ion, or a spin-trapping reagent.
- 25 7. The antibody of claim 1, wherein said antibody is linked to a therapeutic reagent.
8. The antibody of claim 7, wherein the therapeutic reagent is a cytokine, a chemotherapeutic, a radiotherapeutic, a hormone, an antibody Fc fragment, a TLR agonist, a CpG-containing molecule, or an immune co-stimulatory molecule.
- 30 9. The antibody of claim 1, wherein said antibody is a mouse antibody.
10. The antibody of claim 1, wherein said antibody is a humanized antibody.

11. A nucleic acid encoding the heavy chain CDRs of SEQ ID NOS:3, 5 and 7.
12. The nucleic acid of claim 11, wherein said nucleic acid encodes the heavy chain variable region of SEQ ID NO:9 or 25.
- 5 13. The nucleic acid of claim 11, further comprising a nucleic acid segment encoding a non-T-cell peptide or polypeptide.
14. The nucleic acid of claim 11, further comprising a promoter sequence positioned 5' to
10 the nucleic acid encoding the heavy chain CDRs.
15. The nucleic acid of claim 14, wherein said promoter is active in eukaryotic cells.
16. The nucleic acid of claim 14, wherein said promoter is active in prokaryotic cells.
- 15 17. The nucleic acid of claim 11, wherein said nucleic acid is located in a replicable vector.
18. The nucleic acid of claim 17, wherein said replicable vector is a non-viral vector.
- 20 19. The nucleic acid of claim 17, wherein said replicable vector is a viral vector.
20. The nucleic acid of claim 11, further comprising linker-encoding segments, wherein
25 said linker-encoding segments located between said CDR-encoding segments.
21. The nucleic acid of claim 20, wherein one or more of said linker-encoding segments
encode a helix-turn-helix motif.
22. An artificial antibody comprising a heavy chain-encoding segment comprising CDRs
30 comprising the sequences of SEQ ID NOS:3, 5 and 7; and comprising a light chain-
encoding segmetn comprising CDRs comprising the sequences of SEQ ID NOS:15,
19 and 23.
23. The artificial antibody of claim 22, wherein said CDRs are joined by synthetic linkers.

24. The artificial antibody of claim 22, wherein said heavy chain is fused to a non-antibody peptide or polypeptide segment.
- 5 25. The artificial antibody of claim 22, wherein said antibody is linked to a diagnostic reagent.
26. The artificial antibody of claim 25, wherein said diagnostic reagent is a fluorophore, a chromophore, a dye, a radioisotope, a chemilluminant molecule, a paramagnetic ion, or a spin-trapping reagent.
- 10 27. The artificial antibody of claim 22, wherein said antibody is linked to a therapeutic reagent.
- 15 28. The artificial antibody of claim 27, wherein the therapeutic reagent is a cytokine, a toxin, a chemotherapeutic, a radiotherapeutic, a hormone, an antibody Fc fragment, neutrophil elastase, proteinase 3, a TLR agonist, a CpG-containing molecule, or an immune co-stimulatory molecule.
- 20 29. A nucleic acid encoding the heavy chain variable region of the antibody of claim 22.
30. A method of making an antibody comprising (a) introducing into a host cell (i) a nucleic acid sequence encoding a heavy chain comprising SEQ ID NOS:3, 5 and 7, and (ii) a nucleic acid sequence encoding a light chain comprising SEQ ID NOS:15, 19 and 23; and (b) culturing said host cell under conditions supporting expression of said light and heavy chains.
- 25 31. The method of claim 30, wherein said heavy chain variable region comprises SEQ ID NO:9.
- 30 32. The method of claim 30, further comprising the step of linking said antibody to a diagnostic or therapeutic agent.

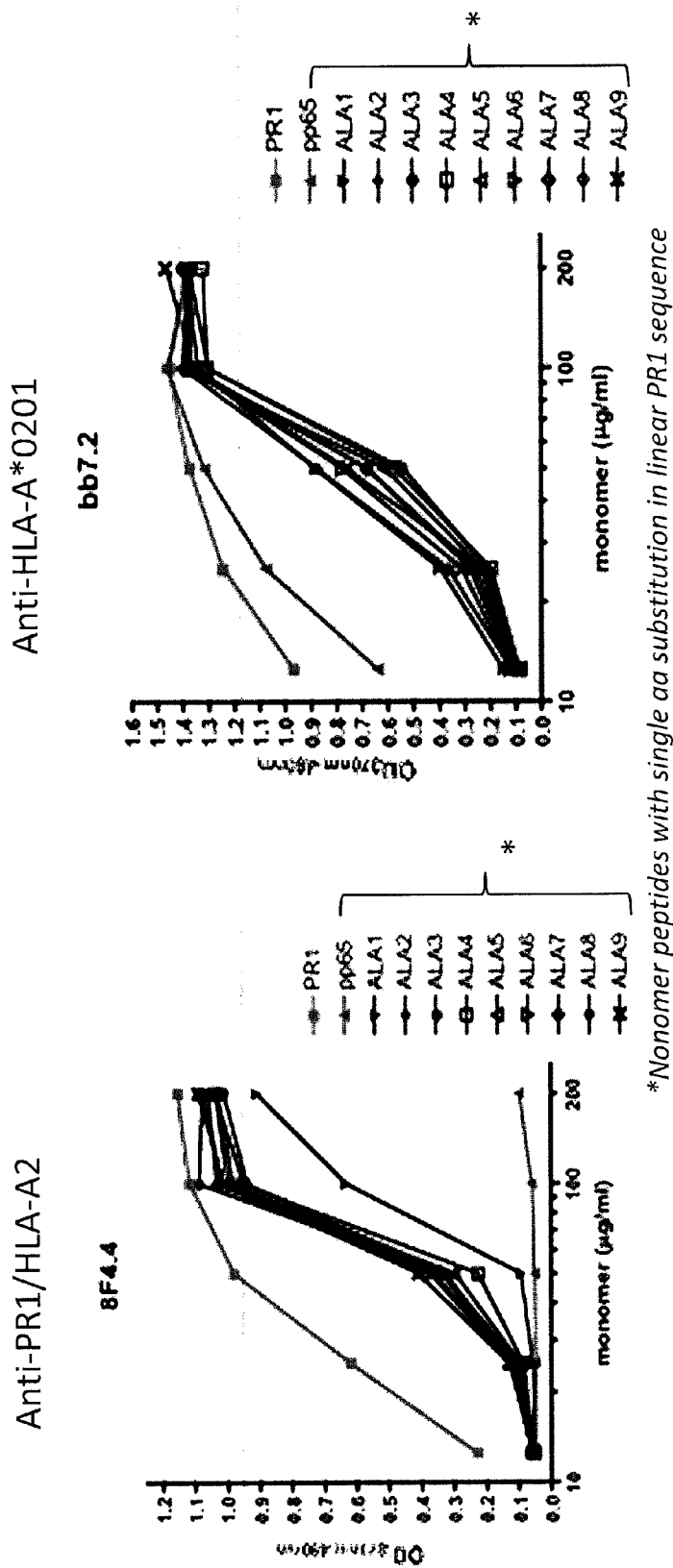
33. A method of detecting abnormal cells in a sample suspected of containing abnormal cells comprising contacting said sample with an antibody of claim 1 or an artificial antibody of claim 22.
- 5 34. The method of claim 33, wherein said antibody or artificial antibody is conjugated to a diagnostic agent.
- 10 35. The method of claim 34, wherein said diagnostic agent is a fluorophore, a chromophore, a dye, a radioisotope, a chemilluminescent molecule, a paramagnetic ion, or a spin-trapping reagent.
36. The method of claim 33, wherein said antibody or artificial antibody is detected using a secondary binding agent.
- 15 37. The method of claim 36, wherein said secondary binding agent is an anti-Fc receptor antibody.
- 20 38. The method of claim 33, wherein said sample is (a) a tumor tissue from head & neck, brain, esophagus, breast, lung, liver, spleen, stomach, small intestine, large intestine, rectum, ovary, uterus, cervix, prostate, testicle or skin tissue, or (b) a fluid such as blood, lymph, urine, bone marrow aspirate or nipple aspirate.
39. The method of claim 33, wherein said sample is from a resected tumor bed.
- 25 40. The method of claim 33, further comprising making a treatment decision based on the presence, absence or degree of detection.
41. The method of claim 40, wherein the treatment decision comprises treating said subject with a PR-1-based peptide vaccine.
- 30 42. The method of claim 33, wherein primary cancer cells, metastatic cancer cells or myeloid dysplastic cells are detected.

43. A method of treating a subject with cancer comprising administering to said subject an antibody of claim 1 or an artificial antibody of claim 22.
- 5 44. The method of claim 43, wherein said antibody or artificial antibody is conjugated to a therapeutic agent.
45. The method of claim 43, wherein said cancer is a solid tumor.
- 10 46. The method of claim 45, wherein said solid tumor is a head & neck tumor, a brain tumor, an esophageal tumor, a breast tumor, a lung tumor, a liver tumor, a spleen tumor, and stomach tumor, a small intestinal tumor, a large intestinal tumor, a rectal tumor, an ovarian tumor, a uterine tumor, a cervical tumor, a prostate tumor, a testicular tumor or a skin tumor.
- 15 47. The method of claim 43, wherein said cancer is a blood cancer.
48. The method of claim 47, wherein said blood tumor is a leukemia or lymphoma.
- 20 49. The method of claim 44, wherein said therapeutic agent is a cytokine, a toxin, a chemotherapeutic, a radiotherapeutic, a hormone, an antibody Fc fragment, a TLR agonist, a CpG-containing molecule, or an immune co-stimulatory molecule.
- 25 50. The method of claim 43, further comprising providing said subject with a second anti-cancer therapy.
51. The method of claim 50, wherein said second anti-cancer therapy is a gene therapy, a chemotherapy, a radiotherapy, a hormone therapy, a toxin therapy or surgery.
- 30 52. The method of claim 43, wherein said antibody or artificial antibody is administered to said subject more than once.
53. The method of claim 43, wherein said cancer is recurrent or metastatic cancer.

54. A method of treating a subject with an autoimmune disease comprising administering to said subject an antibody of claim 1 or an artificial antibody of claim 22.
55. The method of claim 54, wherein said autoimmune disease is Wegener's granulomatosis, Churg-Strauss Syndrome, or systemic small vessel vasculitis.
56. The method of claim 54, wherein said antibody or artificial antibody is conjugated to a therapeutic agent.
57. The method of claim 56, wherein said therapeutic agent is a toxin or apoptosis-inducing agent.
58. The method of claim 54, further comprising providing said subject with a second anti-autoimmune therapy.
59. The method of claim 58, wherein said second anti-autoimmune therapy is an anti-inflammatory agent.
60. The method of claim 54, wherein said antibody is administered to said subject more than once.
61. A purified and isolated nucleic acid segment encoding a CDR comprising the amino sequence of SEQ ID NO:3.
62. The purified and isolated nucleic acid segment of claim 61, wherein the nucleotide sequence comprises SEQ ID NO:2.
63. A purified and isolated nucleic acid segment encoding a CDR comprising the amino sequence of SEQ ID NO:5.
64. The purified and isolated nucleic acid segment of claim 63, wherein the nucleotide sequence comprises SEQ ID NO:4.

65. A purified and isolated nucleic acid segment encoding a CDR comprising the amino sequence of SEQ ID NO:7.
- 5 66. The purified and isolated nucleic acid segment of claim 65, wherein the nucleotide sequence comprises SEQ ID NO:6.
67. A purified and isolated nucleic acid segment encoding a heavy chain variable region comprising the amino sequence of SEQ ID NO:9.
- 10 68. The purified and isolated nucleic acid segment of claim 67, wherein the nucleotide sequence comprises SEQ ID NO:8.
- 15 69. A method of treating a subject with a myeloid dysplastic disease comprising administering to said subject the antibody of claim 1 or the artificial antibody of claim 22.
70. A method of inducing complement-mediated cytotoxicity of an HLA-A2 cancer cell comprising contacting said cancer cell with an antibody of claim 1 or an artificial antibody of claim 22.
- 20 71. A purified and isolated nucleic acid segment encoding a CDR comprising the amino sequence of SEQ ID NO:15.
- 25 72. The purified and isolated nucleic acid segment of claim 71, wherein the nucleotide sequence comprises SEQ ID NO:14.
73. A purified and isolated nucleic acid segment encoding a CDR comprising the amino sequence of SEQ ID NO:19.
- 30 74. The purified and isolated nucleic acid segment of claim 73, wherein the nucleotide sequence comprises SEQ ID NO:18.
75. A purified and isolated nucleic acid segment encoding a CDR comprising the amino sequence of SEQ ID NO:23.

76. The purified and isolated nucleic acid segment of claim 75, wherein the nucleotide sequence comprises SEQ ID NO:22.
- 5 77. A purified and isolated nucleic acid segment encoding a heavy chain variable region comprising the amino sequence of SEQ ID NO:24.
78. The purified and isolated nucleic acid segment of claim 77, wherein the nucleotide sequence comprises the first 324 bases of SEQ ID NO:11.
- 10 79. A nucleic acid encoding the light chain CDRs of SEQ ID NOS:15, 19 and 23.
80. The nucleic acid of claim 79, wherein said nucleic acid encodes the light chain variable region of SEQ ID NO:24.
- 15 81. The nucleic acid of claim 79, further comprising a promoter sequence positioned 5' to the nucleic acid encoding the light chain CDRs.
82. The nucleic acid of claim 81, wherein said promoter is active in eukaryotic cells.
- 20 83. The nucleic acid of claim 81, wherein said promoter is active in prokaryotic cells.
84. The nucleic acid of claim 79, wherein said nucleic acid is located in a replicable vector.
- 25 85. The nucleic acid of claim 84, wherein said replicable vector is a non-viral vector.
86. The nucleic acid of claim 84, wherein said replicable vector is a viral vector.
- 30 87. The nucleic acid of claim 79, further comprising linker-encoding segments, wherein said linker-encoding segments located between said CDR-encoding segments.
88. The nucleic acid of claim 87, wherein one or more of said linker-encoding segments encode a helix-turn-helix motif.



PR1 sequence:

V-L-Q-E-L-N-V-T-V

1-2-3-4-5-6-7-8-9

Fig. 1

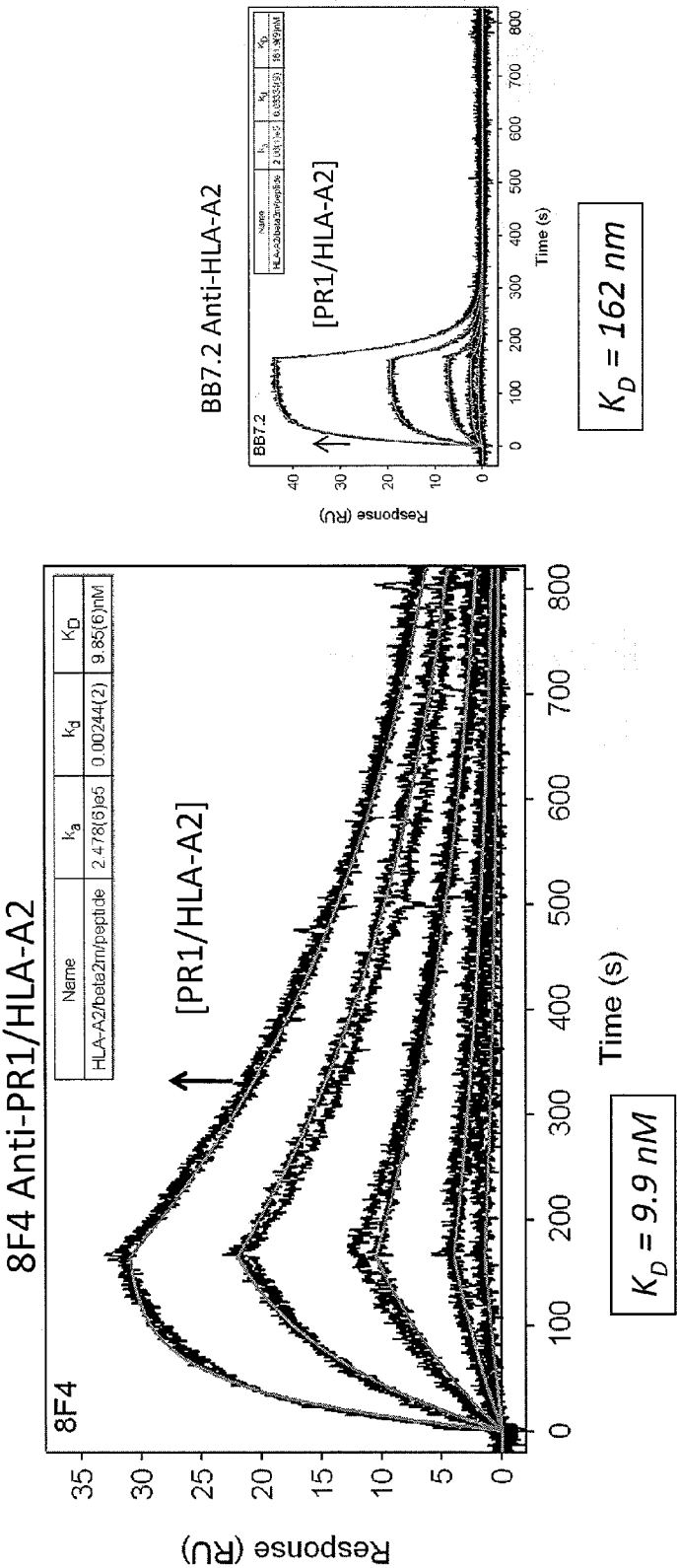


Fig. 2

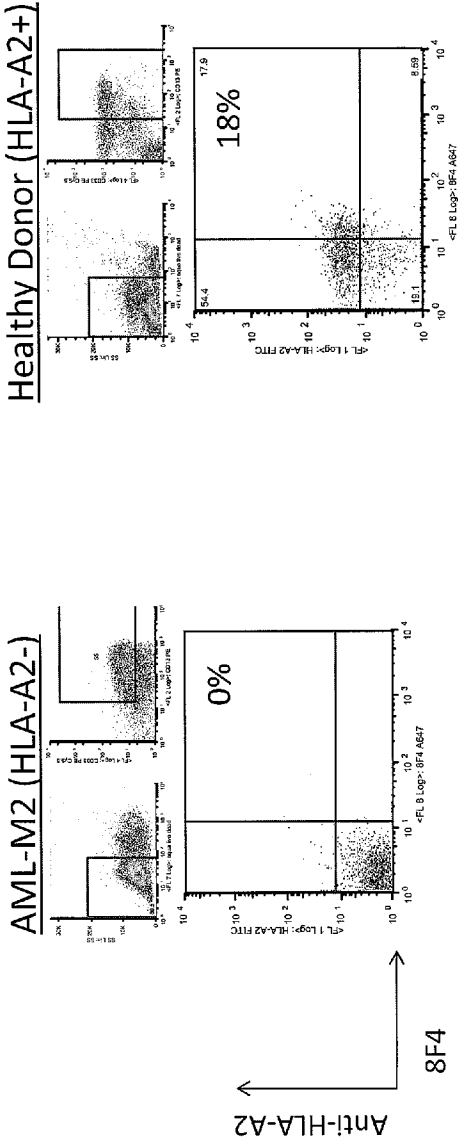


FIG. 3-1

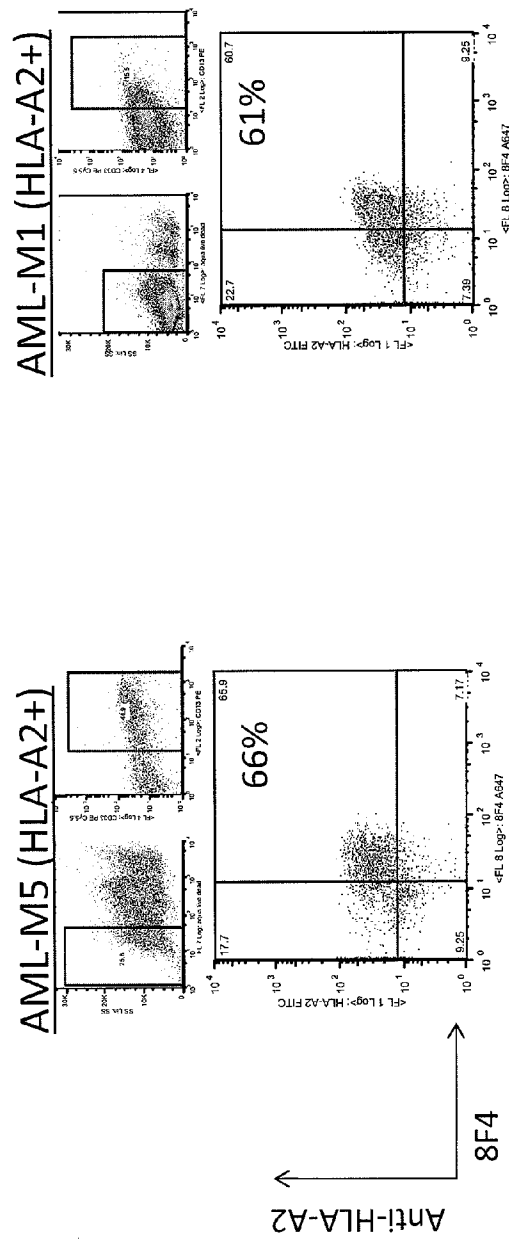
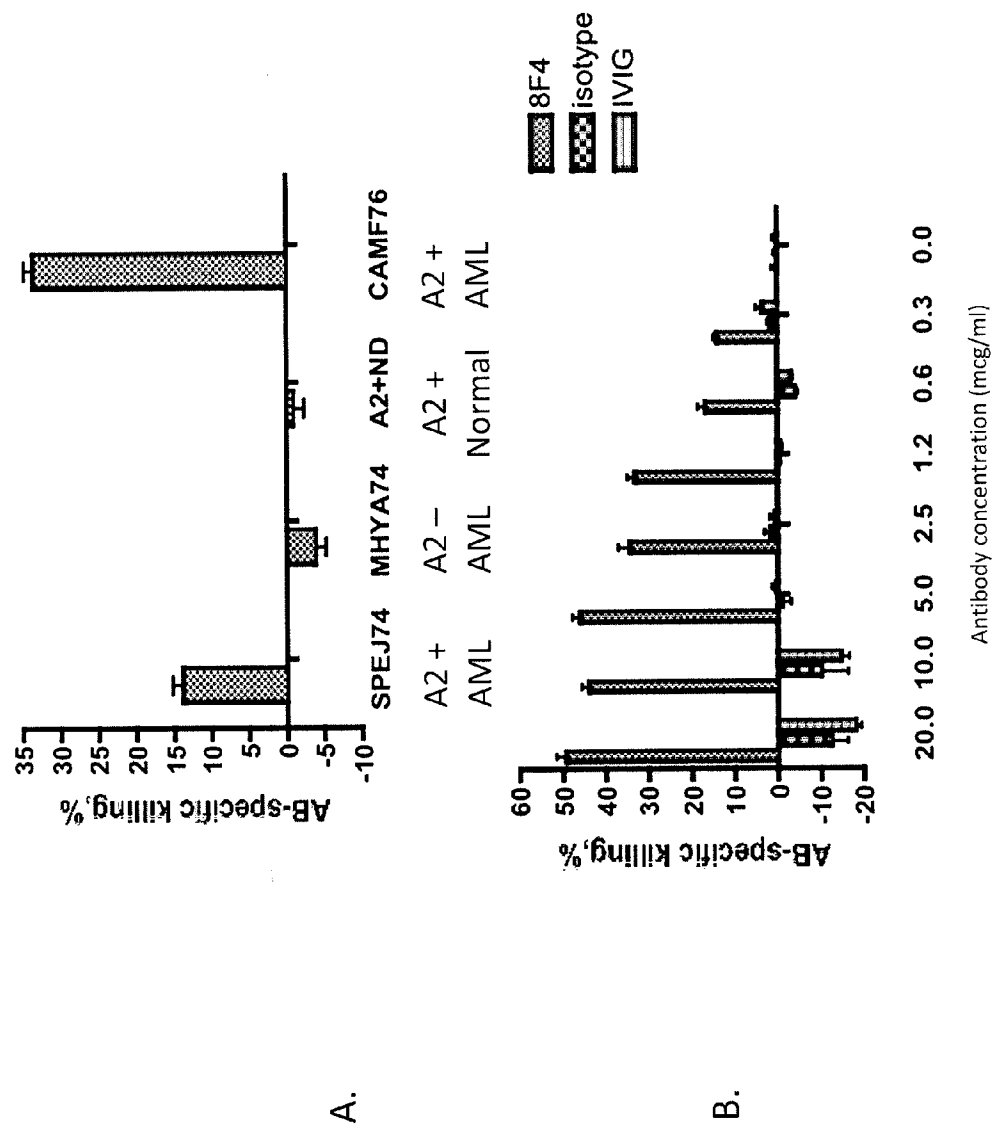


Fig. 3-2



Figs. 4A-4B

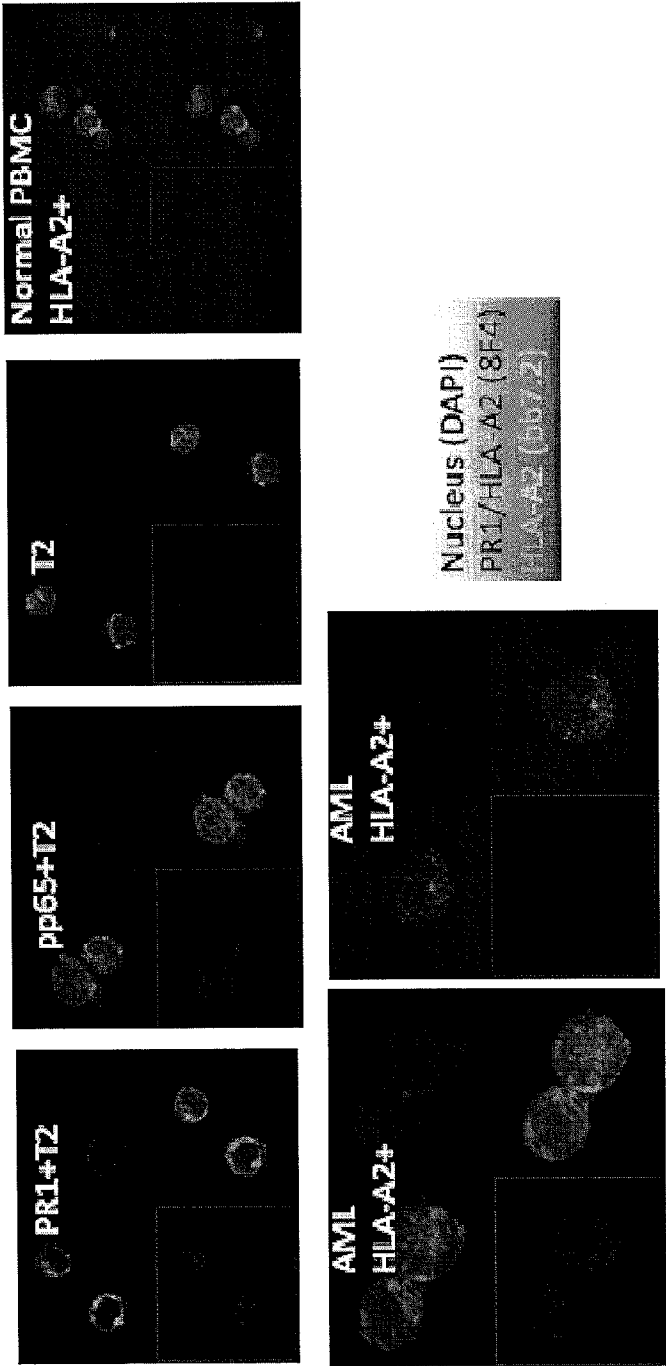
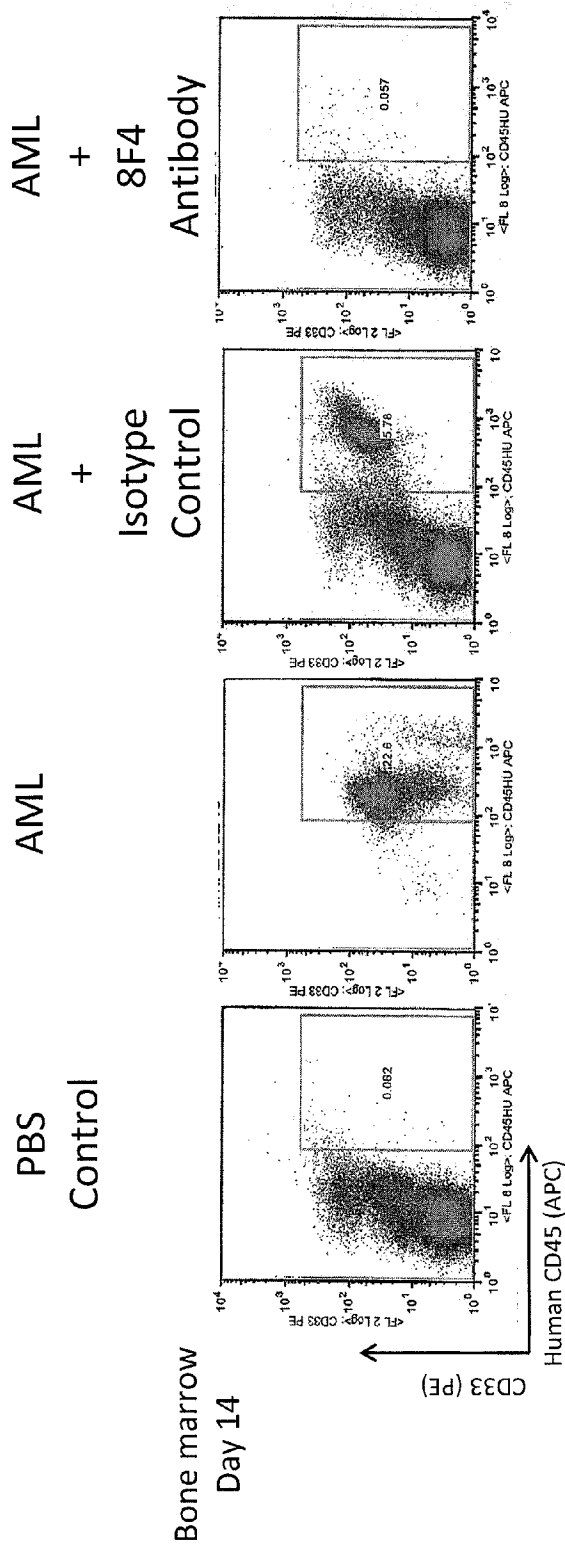


Fig. 5



Patient-derived AML blasts into irradiated A2-transgenic NOD/SCID mice

Fig. 6

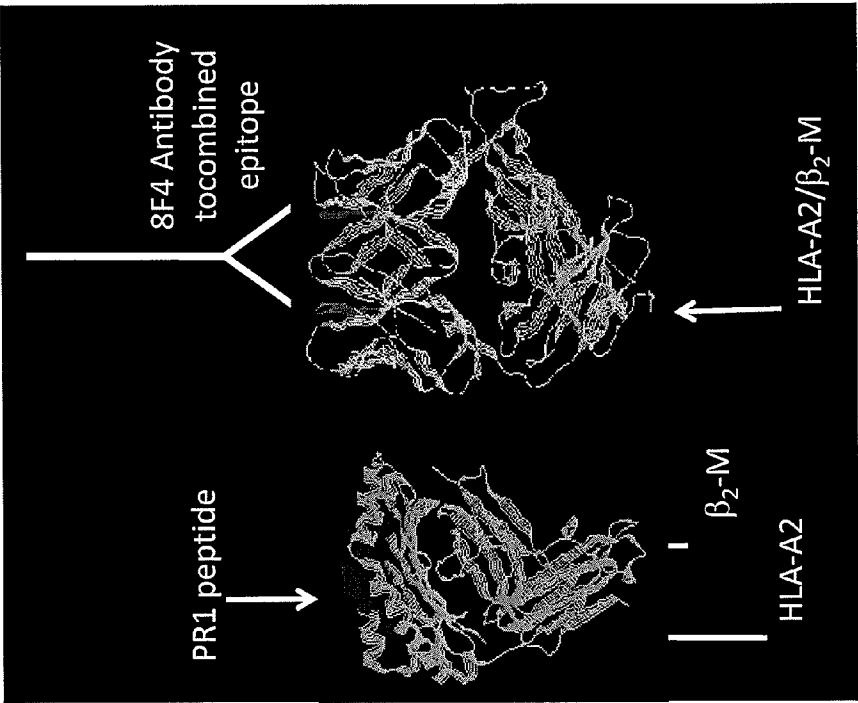


Fig. 7

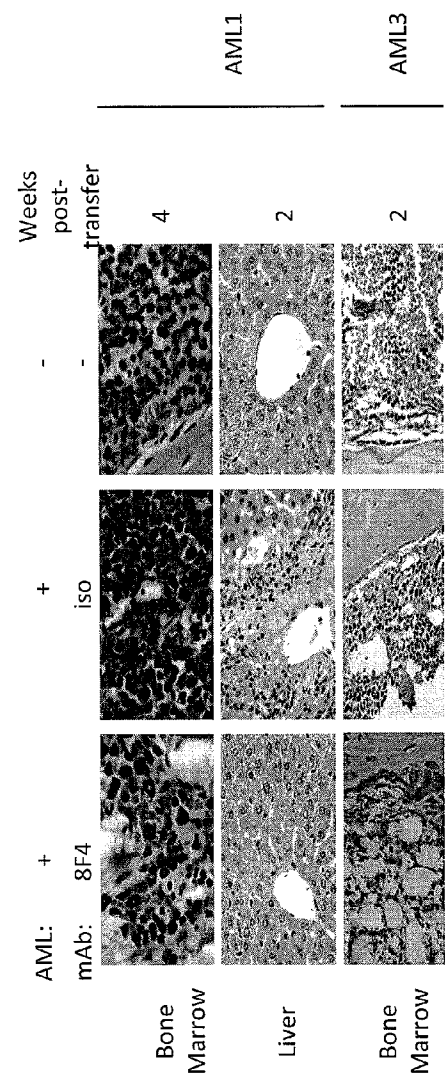


Fig. 8A

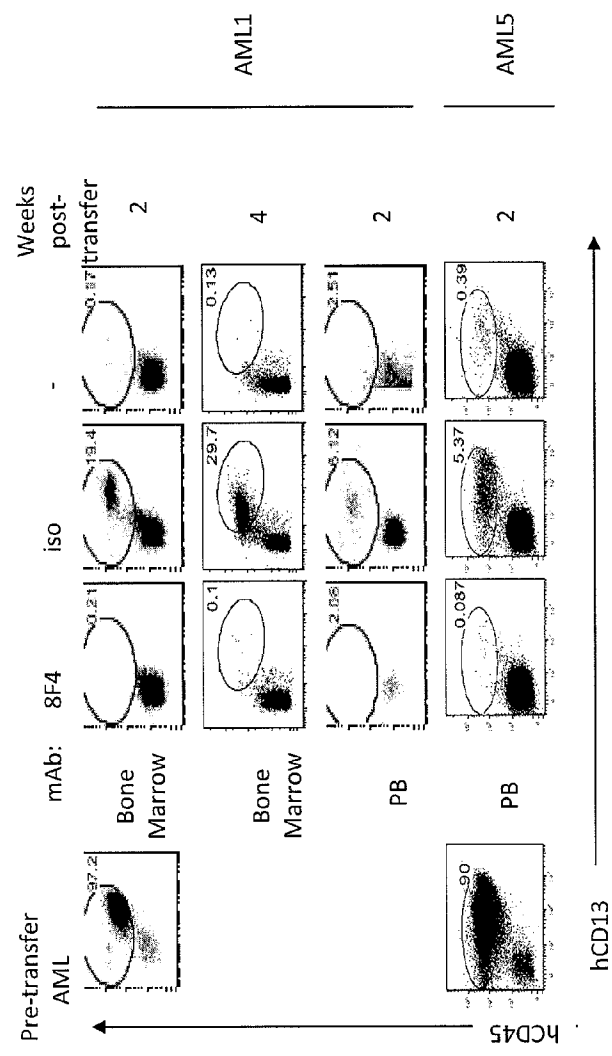
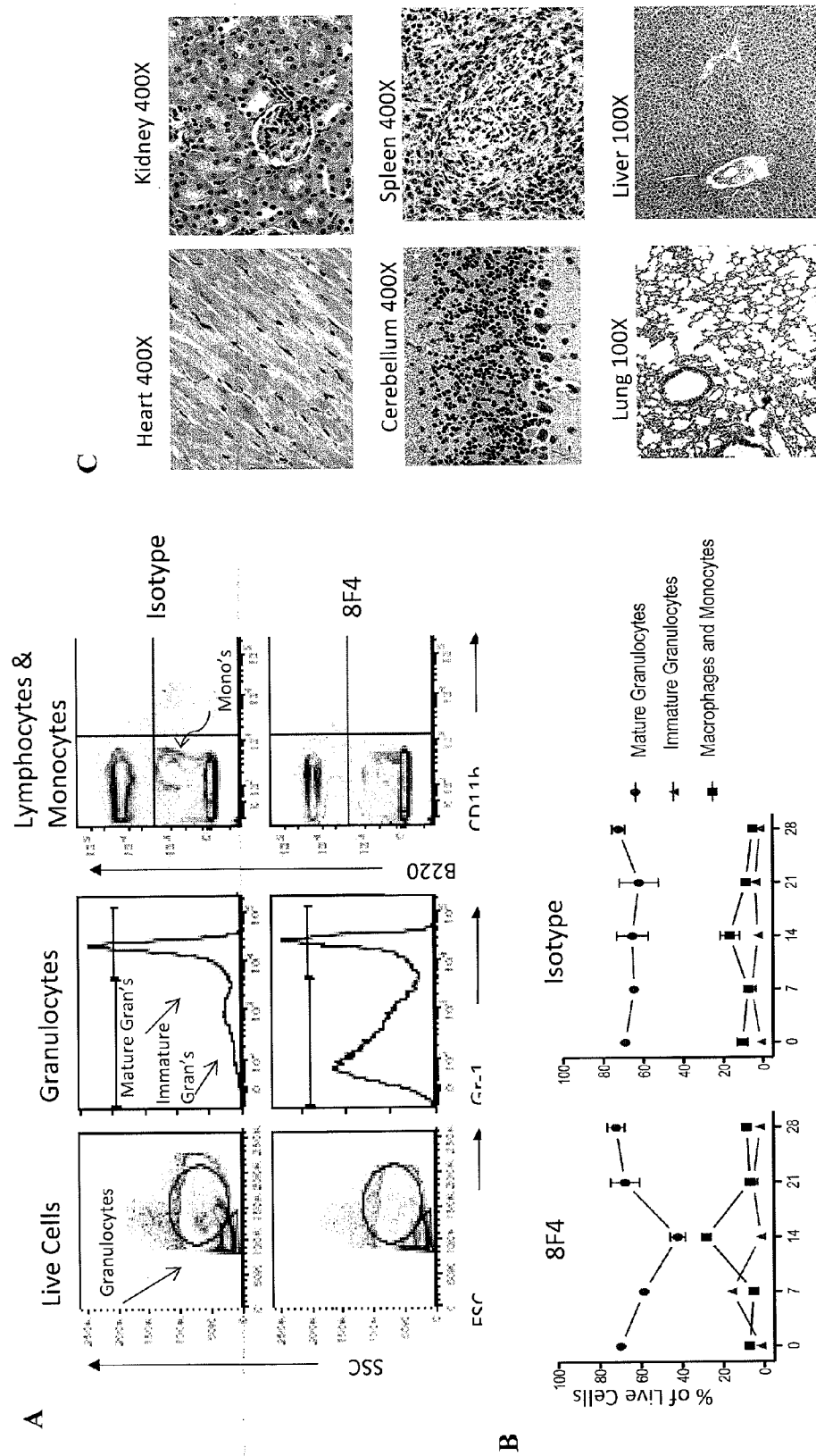
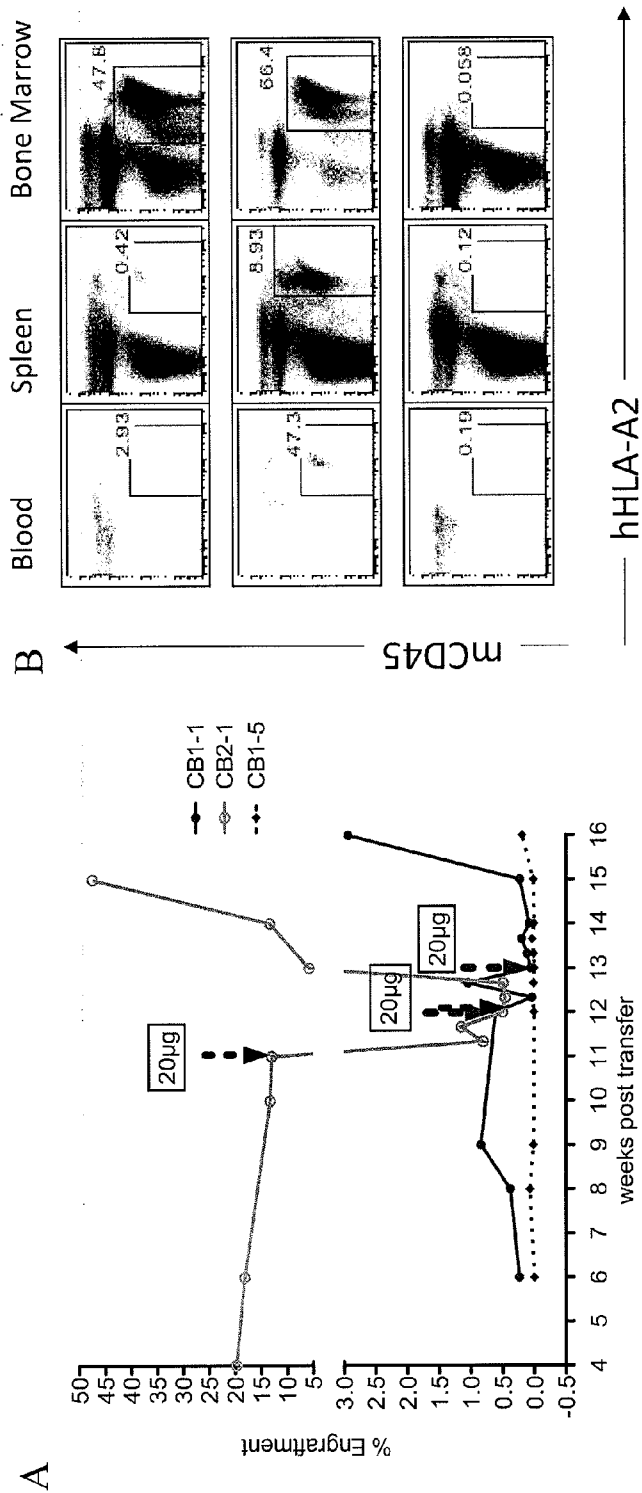


Fig. 8B



Figs. 9A-9C



Figs. 10A-10B