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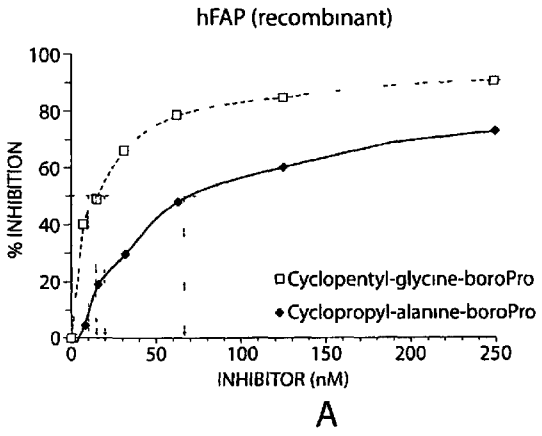
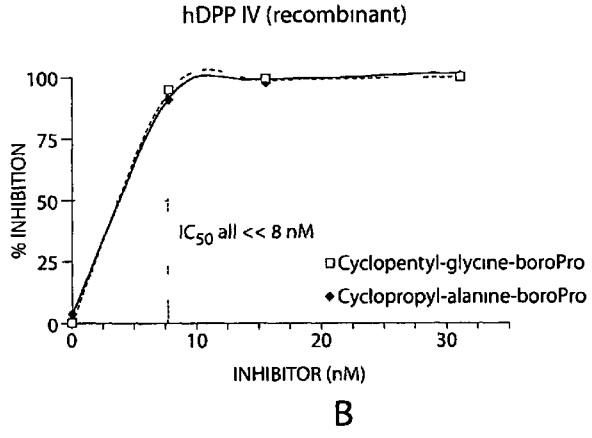
[Continued on next page]

(54) Title: DPP INHIBITORS AND USES THEREOF





(57) Abstract: The invention provides, in part, amino boronic dipeptide agents, compositions thereof and methods of using the amino boronic dipeptide compositions in the treatment of disor ders characterized by abnormal cell proliferation, cardiovascular disorders, and infectious diseases

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DPP INHIBITORS AND USES THEREOF

Field of the Invention

This invention relates to dipeptidyl dipeptidase inhibitors (such as DPP-IV inhibitors) and methods of use thereof in the treatment of disease.

Background of the Invention

Cancer is the second leading cause of death, resulting in one out of every four deaths, in the United States. In 1997, the estimated total number of new diagnoses for lung, breast, prostate, colorectal and ovarian cancer was approximately two million. Due to the ever increasing aging population in the United States, it is reasonable to expect that rates of cancer incidence will continue to grow.

Cancer is currently treated using a variety of modalities including surgery, radiation therapy and chemotherapy. The choice of treatment modality will depend upon the type, location and dissemination of the cancer. One of the advantages of surgery and radiation therapy is the ability to control to some extent the impact of the therapy, and thus to limit the toxicity to normal tissues in the body. Chemotherapy is arguably the most appropriate treatment for disseminated cancers such as leukemia and lymphoma as well as metastases. Chemotherapy is generally administered systemically and thus toxicity to normal tissues is a major concern. Not all tumors, however, respond to chemotherapeutic agents and others, although initially responsive to chemotherapeutic agents, may develop resistance. As a result, the search for effective anti-cancer drugs has intensified in an effort to find even more effective agents with less non-specific toxicity.

Recently, much emphasis has been placed on the use of immunotherapy for the treatment and prevention of cancer and other disorders, including infectious disease. Immunotherapy provides the cell specificity that other treatment modalities lack. Methods for enhancing the efficacy of immune based therapies would be beneficial.

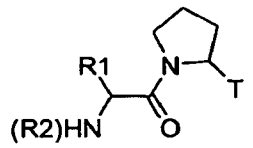
Summary of the Invention

The invention provides, in part, amino boronic agents, compositions thereof and methods of using the amino boronic agents (and compositions thereof) in the treatment (including prevention) of, inter alia, disorders characterized by abnormal cell proliferation

(e.g., cancer), cardiovascular disorders, and infectious diseases. In some embodiments, the amino boronic acid agents are dipeptides or analogs thereof, although they are not so limited.

The invention provides the agents of Formulae I-XIII (a through e). These agents may be provided in an isolated form. These agents may also be provided in compositions. As used herein, the terms agent and compound are used interchangeably.

Some agents of the invention share the common structure of **Formula Ia**

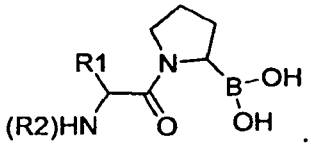


wherein T is a reactive group such as an organo boronate, an organo phosphonate, a fluoroalkylketone, an halomethyl ketone, a diazomethyl ketone, a dimethylsulphonium salt, an alphaketo carboxylic acid, an alphaketo ester, an alphaketo amide, an alpha-diketone, an acyloxymethyl ketone, an aldehyde, an epoxysuccinyl, an N-peptidyl-O-acylhydroxylamine, an azapeptide, a fluoroolefin, a peptidyl (alpha-aminoalkyl) phosphonate ester, or a nitrile. R1 in its broadest sense may be a hydrophobic or branched side chain of an amino acid or amino acid analog (e.g., a non-naturally occurring amino acid), or it may be an amino acid or amino acid analog (e.g., a non-naturally occurring amino acid) that comprises at least 3 carbon atoms. In certain embodiments, R1 comprises one or more ring structures or — R3 (i.e., an additional carbon bond from the peptide backbone), wherein R3 comprises one or more ring structures, and R2 is a hydrogen, peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The ring structures may be wholly carbon rings or they may be substituted at one or more positions with for example sulfur, nitrogen, and the like. In some embodiments, R1 comprises a five carbon ring structure. In some embodiments R3 comprises a three carbon ring structure. The proline residue attached to the reactive group is referred to as a pyrrolidine ring. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine. The bond between the carbon in the pyrrolidine ring and T may be in the L or D configuration. The bond between C and R1 may be in the L or D configuration, although in some embodiments, it is preferably in the L configuration.

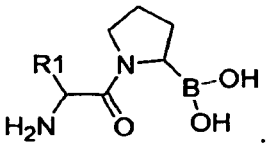
The agents of Formula Ia include agents of **Formula Ib**

wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

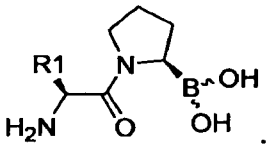
The agents of Formula Ib further include agents of **Formula Ic**



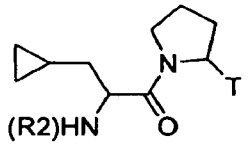
The agents of Formula Ic even further include agents of **Formula Id**



The agents of Formula Id even further include agents of **Formula Ie**

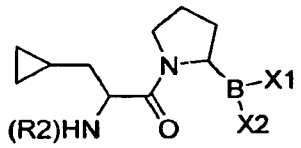


Some agents of the invention have the structure of **Formula Ha**



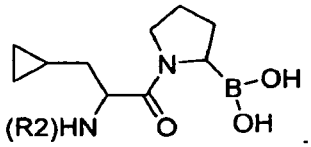
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the L or D configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula Ha include agents of **Formula Hb**

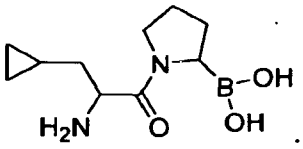


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

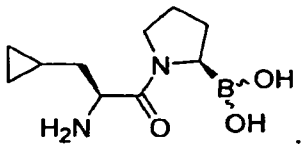
The agents of Formula lib further include agents of **Formula Hc**



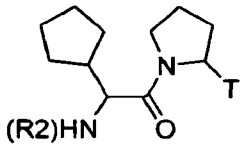
The agents of Formula *Uc* even further include agents of Formula **lid**



The agents of Formula lid even further include agents of **Formula Ue** (referred to herein as cyclopropyl-alanine-boroPro)

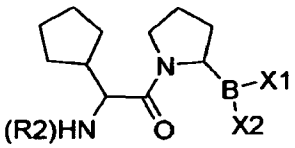


Some agents of the invention have the structure of **Formula HIa**



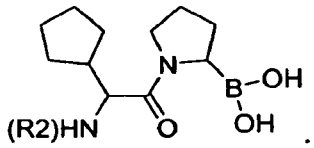
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the L or D configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula Ilia include agents of **Formula HIb**

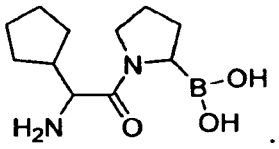


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

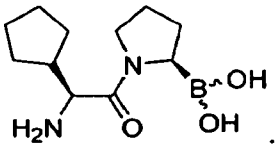
The agents of Formula IIIb further include agents of **Formula IHc**



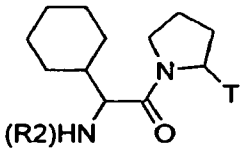
The agents of Formula IIIc even further include agents of **Formula IHd**



The agents of Formula IIId even further include agents of **Formula IHe** (referred to herein as cyclopentyl-glycine-boroPro)

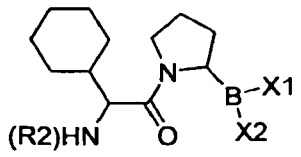


Some agents of the invention have the structure of **Formula IVa**



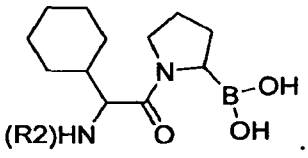
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the L or D configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula IVa include agents of **Formula IVb**

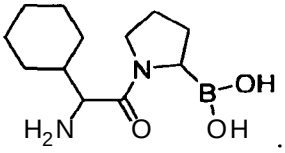


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

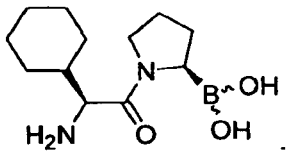
The agents of Formula IVb further include agents of **Formula IVc**



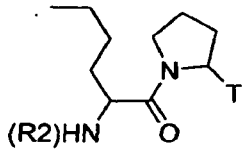
The agents of Formula IVc even further include agents of **Formula IVd**



The agents of Formula IVd even further include agents of **Formula IVe** (referred to herein as cyclohexyl-glycine-boroPro)

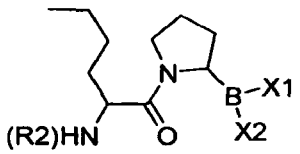


Still other agents of the invention have the structure of **Formula Va**



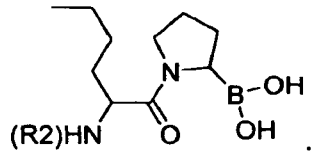
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the L or D configuration. The amino terminal amino acid residue may be in the L or D configuration, although in some embodiments, it is preferably in the L configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula Va include agents of Formula Vb

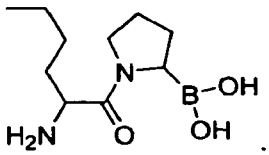


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

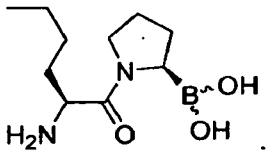
The agents of Formula Vb further include agents of **Formula Vc**



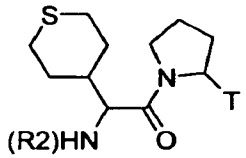
The agents of Formula Vc even further include agents of **Formula Vd**



The agents of Formula Vd even further include agents of **Formula Ve** (referred to herein as Norleu-boroPro)

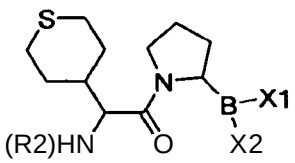


Other agents of the invention have the structure of **Formula Via**



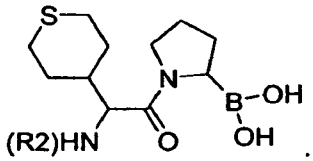
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula Via include agents of **Formula VIb**

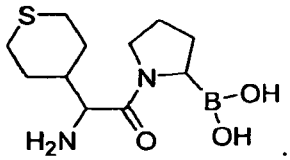


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

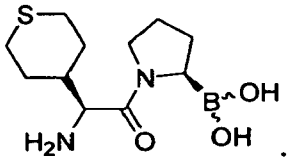
The agents of Formula VIb further include agents of **Formula VIc**



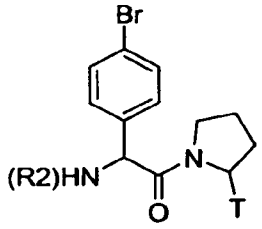
The agents of Formula VIc even further include agents of **Formula VId**



The agents of Formula VId even further include agents of Formula VIe

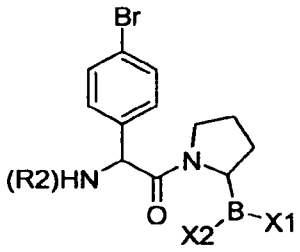


Other agents of the invention have the structure of **Formula Vila**



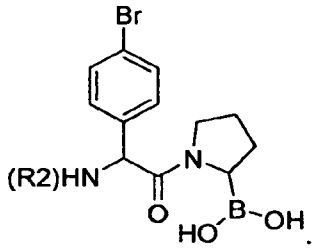
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L conf guration. hi some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula Vila include agents of **Formula VIIb**

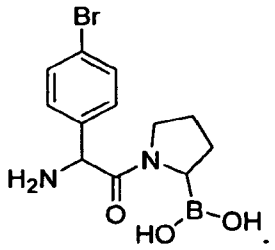


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

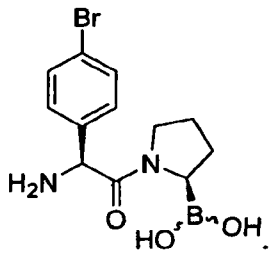
The agents of Formula VIIb further include agents of **Formula VIIc**



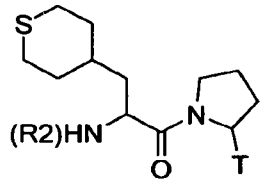
The agents of Formula VIIc even further include agents of **Formula VTId**



The agents of Formula VIId even **further** include agents of **Formula VIIe**

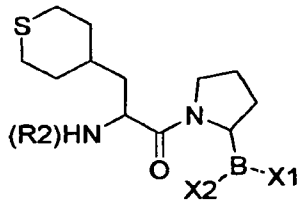


Other agents of the invention have the structure of **Formula Villa**



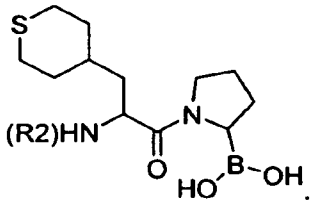
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula Villa include agents of **Formula VlIIb**

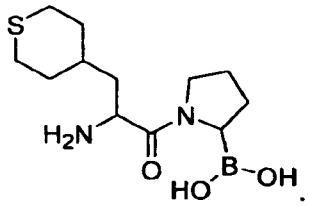


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

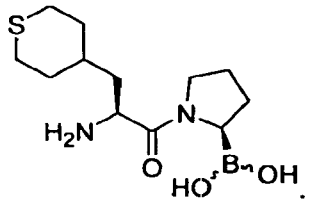
The agents of Formula VIIIb further include agents of **Formula VIIIc**



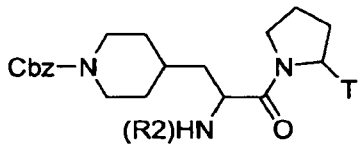
The agents of Formula VIIIc even further include agents of **Formula VTIId**



The agents of Formula VIIId even further include agents of **Formula VIIIe**

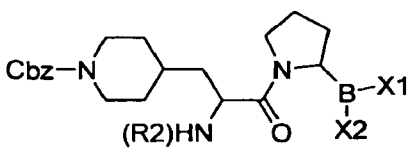


Other agents of the invention have the structure of **Formula IXa**



wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

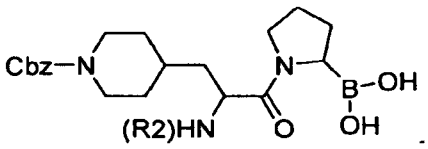
The agents of Formula IXa include agents of **Formula IXb**



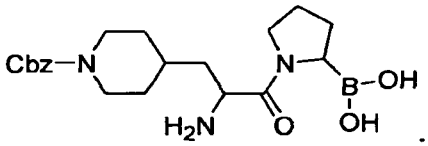
wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH.

The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

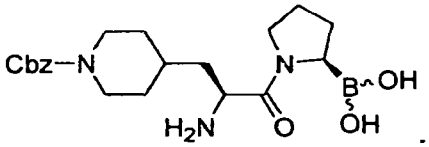
The agents of Formula IXb further include agents of **Formula IXc**



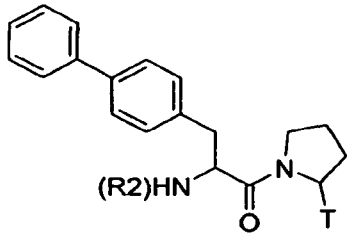
The agents of Formula IXc even further include agents of **Formula IXd**



The agents of Formula IXd even further include agents of **Formula IXe**

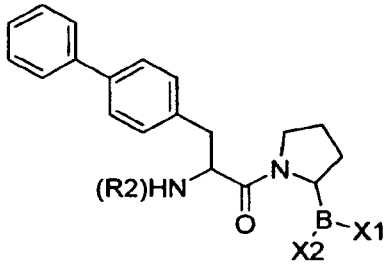


Other agents of the invention have the structure of **Formula Xa**



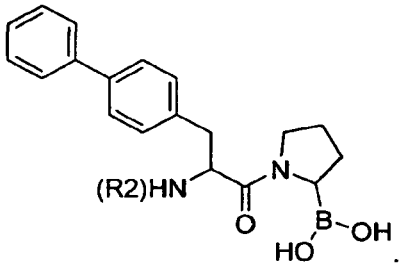
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula Xa include agents of **Formula** Xb

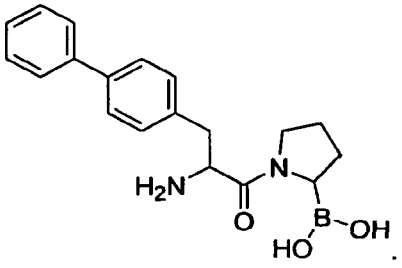


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

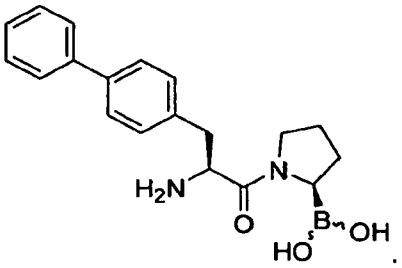
The agents of Formula Xb further include agents of **Formula Xc**



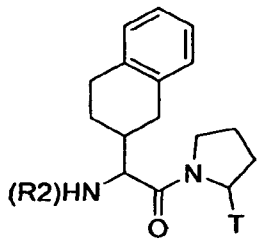
The agents of Formula **Xc** even further include agents of **Formula Xd**



The agents of Formula **Xd** even further include agents of **Formula Xe**

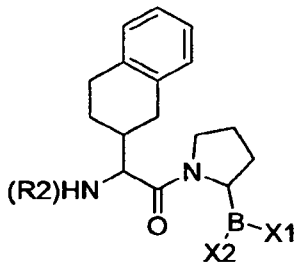


Other agents of the invention have the structure of **Formula XIa**



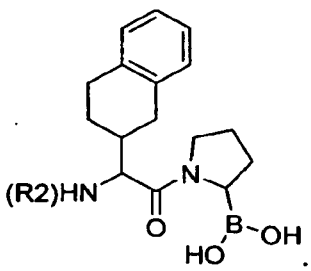
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula XIa include agents of **Formula XIb**

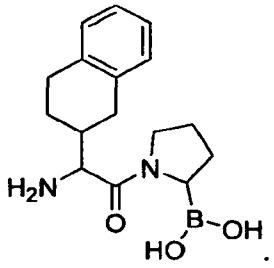


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

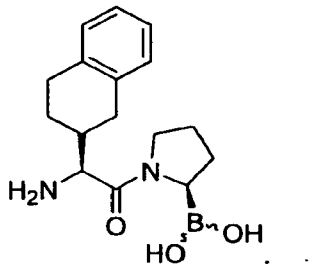
The agents of Formula XIb further include agents of **Formula XIc**



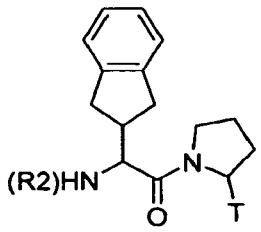
The agents of Formula **XIc** even further include agents of **Formula XId**



The agents of Formula XId even further include agents of **Formula XIe**

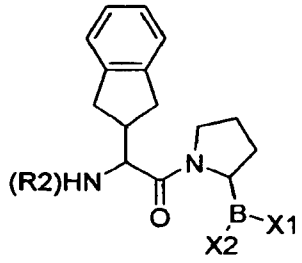


Other agents of the invention have the structure of Formula **XIIa**



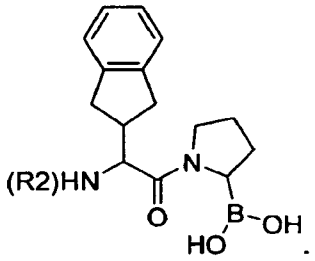
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula XIIa include agents of **Formula XIIb**

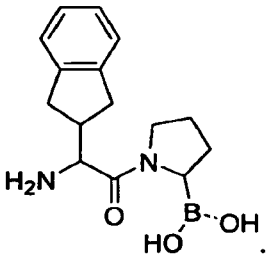


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

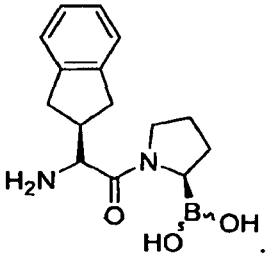
The agents of Formula XIIb further include agents of **Formula XIIc**



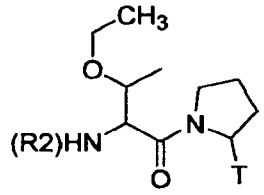
The agents of Formula XIIc even further include agents of **Formula XIId**



The agents of Formula XIId even further include agents of **Formula XIIe**

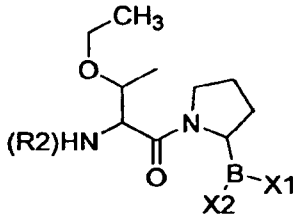


Other agents of the invention have the structure of **Formula XIIIa**



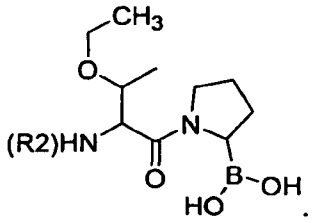
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. The amino terminal amino acid residue may be in the L or D configuration, although in some embodiments, it is preferably in the L configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula XIIIa include agents of **Formula XIIIb**

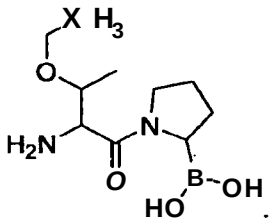


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

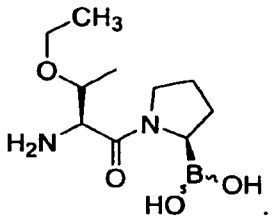
The agents of Formula XIIIb further include agents of **Formula XIIIc**



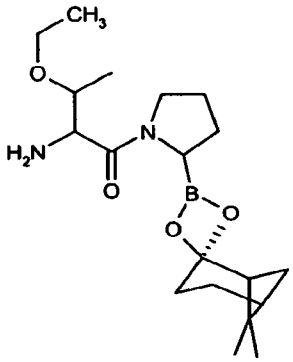
The agents of Formula XIIIc even further include agents of **Formula XIIId**



The agents of Formula XIIId even further include agents of **Formula XIIIe**



An example of an agent that converts to an agent of Formula XIIId when exposed to aqueous solution is represented by **Formula XIIIg**



The composition may further comprise a pharmaceutically acceptable carrier.

The composition may further comprise a second therapeutic agent. The second therapeutic agent may be an antigen, an antibody, an antibody fragment, an anti-microbial agent, an anti-cancer agent, an anti-inflammatory agent, a statin, a COX-2 inhibitor, and the like.

In some embodiments, the antigen is a cancer antigen or a microbial antigen. In some embodiments, the antibody or antibody fragment is a cancer antibody, a cancer antibody fragment, an anti-microbial antibody, or anti-microbial antibody fragment. The anti-cancer antibody or anti-cancer antibody fragment may be an anti-HER2/neu antibody, an anti-HER2/neu antibody fragment, an anti-CD20 antibody, or an anti-CD20 antibody fragment. In

some embodiments, the anti-HER2 antibody is trastuzumab. In some embodiments, the anti-CD20 antibody is rituxan. In some embodiments, the antibody or antibody fragment is conjugated to a toxin, a chemotherapeutic agent or a radioisotope.

In some embodiments, the anti-microbial agent is an anti-bacterial agent, an anti-viral agent, an anti-fungal agent, an anti-parasitic agent or an anti-mycobacterial agent.

In some embodiments, the agent is present in an effective amount.

In some embodiments, the composition is formulated for oral delivery.

The invention provides a method for stimulating an immune response in a subject by administering to a subject in need thereof a composition comprising an agent of the invention (e.g., an agent of Formula I, V or XIII) or a prodrug thereof in an amount effective to stimulate an immune response in the subject.

The immune response may be an antigen-specific immune response, an innate immune response, or an antibody-dependent cell-mediated cytotoxicity.

In some embodiments, the subject has a condition that would benefit from immune stimulation. The condition may be a cancer, an infectious disease, a cardiovascular disorder, or an interferon-gamma (IFN-γ) responsive condition.

The subject may be at risk of developing a cancer or an infectious disease, a cardiovascular disorder, or an IFN-γ responsive condition.

In some embodiments, the composition and the second therapeutic agent are administered by different routes. In some embodiments, the composition and the second therapeutic agent are administered at different times. The agent of the invention may be administered before, at the same time as, and/or after the second therapeutic agent.

In some embodiments, the subject is immunocompromised. The immunocompromised subject may be genetically immunocompromised. The subject may have a genetic deficiency. The genetic deficiency may be severe combined immunodeficiency (SCID), agammaglobulinemia, or congenital disorder of glycosylation (CDG). In some embodiments, the subject has an immunoglobulin deficiency that is common variable immunodeficiency.

In another aspect, the invention provides a method for inhibiting an infectious disease in a subject comprising administering to a subject in need thereof the composition comprising any of the foregoing agents, alone or in combination, in an amount effective to inhibit the infectious disease.

In some embodiments, the subject is at risk of developing an infectious disease. The infectious disease may be a bacterial infection, a viral infection, a fungal infection, a parasitic infection, or a mycobacterial infection.

In some embodiments, the infectious disease is Bacillus anthracis infection (anthrax), Varicella (chicken pox), Clostridium botulinum infection (botulism), Yersinia pestis infection (plague), variola major infection (smallpox), Francisella tularensis infection (tularemia), Ebola infection (e.g., Ebola virus hemorrhagic fever), Marburg infection (e.g., Marburg virus hemorrhagic fever), Arenaviruses (e.g., Lassa infection, Lassa fever, Machupo infection), tuberculosis, or Junin infection.

In some other preferred embodiments, the infectious disease is Brucella species infection (brucellosis), Burkholderia mallei infection (glanders), Burkholderia pseudomallei infection (melioidosis), Chlamydi psittaci infection (psittacosis), Clostridium perfringens infection (Epsilon toxin), Coxiella burnetii infection (Q fever), nipah virus infection, hantavirus infection, Escherichia coli O157:H7 infection (E. coli), Ricinus communis infection (Ricin toxin), Rickettsia prowazekii infection (typhus fever), Salmonella species infection (salmonellosis), Salmonella Typhi infection (typhoid fever), Shigella infection (shigellosis), Vibrio cholerae infection (cholera), alphavirus infection (e.g., Venezuelan equine encephalitis, eastern equine encephalitis, and western equine encephalitis), or filoviruses infection(s).

The bacterial infection may be E. coli infection, Staphylococcus infection, Streptococcus infection, Pseudomonas infection, Legionella infection, Pneumococcus infection, Enterobacter infection, Salmonella infection, Listeria infection, or Pasteurella infection.

The viral infection may be HIV infection, Herpes simplex virus infection, cytomegalovirus infection, hepatitis infection, human papilloma virus infection, Epstein Barr virus infection, adenovirus infection, influenza virus infection, respiratory syncytial virus infection, varicella-zoster virus infection, small pox infection, monkey pox infection, or SARS infection.

The fungal infection may be candidiasis, ringworm infection, histoplasmosis, blastomycosis, paracoccidioidomycosis, crytococcosis, aspergillosis, chromomycosis, mycetoma infections, pseudallescheriasis, or tinea versicolor infection.

The parasitic infection may be amebiasis, Trypanosoma cruzi infection, Fascioliasis, Leishmaniasis, Plasmodium infection, Onchocerciasis, Paragonimiasis, Trypanosoma brucei infection, Pneumocystis, Trichomonas vaginalis infection, Taenia infection, Hymenolepsis, Echinococcus infection, Schistosomiasis, neurocysticercosis, Necator americanus infection or Trichuris trichuria infection.

The mycobacterial infection may be Mycobacterium tuberculosis infection or Mycobacterium leprae infection.

In some embodiments, the composition is administered prior to the antigen, antibody or antibody fragment. In other embodiments, the composition is administered after the anti-microbial agent.

In another aspect, the invention provides a method for treating cancer in a subject comprising administering to a subject in need thereof any of the foregoing agents, alone or in combination, in an amount effective to treat the cancer.

In some embodiments, the method further comprises administering to the subject a second therapeutic agent. The second therapeutic agent may be an antigen, an antibody, an antibody fragment, an anti-cancer agent such as a chemotherapy, or an enzyme inhibitor. The antigen may be a cancer antigen. The antibody or antibody fragment may be an anti-cancer antibody or an anti-cancer antibody fragment. The anti-cancer antibody or anti-cancer antibody fragment may be an anti-HER2/neu or anti-CD20 antibody or an antibody fragment thereof.

The anti-cancer agent may be docetaxel (TAXOTERE), cisplatin, gemcitabine, pemetrexed (ALIMTA), erlotinib (TARCEVA), gefitinib (IRESSA), temozolomide (TEMODAR), carboplatin, cyclophosphamide or doxorubicin. The enzyme inhibitor may be a tyrosine kinase inhibitor, a CDK inhibitor, a MAP kinase inhibitor, or an EGFR inhibitor.

The cancer may be non-small cell lung carcinoma, melanoma, pancreatic cancer, breast cancer, renal cell carcinoma, chronic lymphocytic leukemia, or Non-Hodgkin's lymphoma. In some embodiments, the cancer is a refractive cancer.

The method may further comprise administering to the subject an anti-cancer therapy. The anti-cancer therapy may be radiation or surgery.

In some embodiments, the composition is administered following the anti-cancer therapy. In some embodiments, the composition and the second therapeutic agent are administered by different routes. The composition and the second therapeutic agent are

administered at different times. The agent of the invention may be administered before, at the same time as, and/or after the second therapeutic agent.

In another aspect, the invention provides a method for preventing anti-microbial drug resistance in a subject in need thereof the composition comprising an agent of the invention (e.g., an agent of Formula I, V or XIII) or a prodrug thereof in an amount effective to reduce the risk of resistance to the anti-microbial agent.

In yet another aspect, the invention provides a method of shortening a vaccination course comprising administering to a subject in need of immunization the composition comprising an agent of the invention (e.g., an agent of Formula I, V or XIII) or a prodrug thereof in an amount effective to induce an antigen-specific immune response to a vaccine administered in a vaccination course, wherein the vaccination course is shortened by at least one immunization or at least one day.

In some embodiments, the vaccine is a hepatitis vaccine, a mumps vaccine, a measles vaccine, a rubella vaccine, a flu vaccine, a polio vaccine, a tetanus vaccine, a DTaP vaccine, an HiB vaccine, a Pneumococcus vaccine, a MMR vaccine, a varicella vaccine, a DPT vaccine, or a Td vaccine.

In another aspect, the invention provides a method for stimulating hematopoiesis in a subject comprising administering to a subject in need thereof the composition comprising an agent of the invention (e.g., an agent of Formula I, V or XIII) or a prodrug thereof in an effective amount to increase the number of hematopoietic cells or mature blood cells in the subject.

In some embodiments, the subject was exposed or will be exposed to a hematopoietic cell inhibitor. In some embodiments, the subject may be neutropenic or anemic.

In some embodiments, the method further comprises collecting hematopoietic cells from the subject after administration of the composition, and reintroducing the collected cells into the subject.

In another aspect, the invention provides a method for stimulating growth factor production by stromal cells comprising contacting stromal cells with the composition comprising an agent of the invention (e.g., an agent of Formula I, V or XIII) or a prodrug thereof in an amount effective to stimulate growth factor production. The contacting may occur *in vitro*. In some embodiments the growth factor is G-CSF. In other embodiments, the growth factor is a chemokine. The chemokine may be IL-8 or KC/CXCL1 .

In some embodiments, the method further comprises culturing hematopoietic progenitor cells in the presence of the stromal cells.

These and other aspects of the invention will be described in greater detail below. Throughout this disclosure, all technical and scientific terms have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains unless defined otherwise.

Each of the limitations of the invention can encompass various embodiments of the invention. It is, therefore, anticipated that each of the limitations of the invention involving any one element or combinations of elements can be included in each aspect of the invention.

Brief Description of the Figures

FIGs. IA-B are graphs showing inhibition of human FAP (A) and human DPP-TV (B) by cyclopropyl-alanine-boroPro and cyclopentyl-glycine-boroPro.

FIGs. 2A-D are graphs showing inhibition of DPP-IV (A), DPP 8 (B), FAP (C), and DPP 2 (D) by Norleu-boroPro.

FIGs. 3A-B are histograms showing induction of IL-8 (A) and G-CSF (B) by human bone marrow stromal cells *in vitro* by cyclopropyl-alanine-boroPro and cyclopentyl-glycine-boroPro.

FIGs. 4A-D are graphs showing inhibition induction of human IL-I beta (A and D), human G-CSF (B), and mouse CXCLl /KC (C) by cyclopentyl-glycine-boroPro.

FIGs. 5A-B are graphs showing inhibition of serum DPP-IV activity (A) and induction of serum CXCL1/KC (B) by cyclopropyl-alanine-boroPro and cyclopentyl-glycine-boroPro.

FIGs. 6A-B are graphs showing inhibition of serum DPP-IV activity (A) and induction of serum CXCL1/KC (B) by Norleu-boroPro administered orally or subcutaneously.

FIGs. 7A-B are histograms showing induction of IFN-gamma from splenocytes by cyclopentyl-glycine-boroPro (A) and cyclohexyl-glycine-boroPro (B).

FIG. 8 is a table showing the structures of various DPP inhibitors and the DPP8 and DPP9 inhibition profile of each.

FIGs. 9A-H are graphs showing caspase activation by inhibitor VIe (A), VIIe (B), VIIIe (C), IXe (D), Xe (E), XIe (F), XIIe (G), and XIIIe (H).

It is to be understood that the Figures are not required for enablement of the invention.

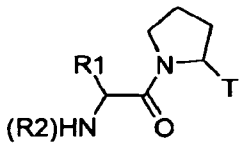
Detailed Description of the Invention

The invention relates, in part, to particular agents and prodrugs thereof useful for inhibiting dipeptidyl peptidases such as DPP-IV, and to methods of use thereof in the treatment of particular diseases and/or in the stimulation of immunity in a subject. The diseases include abnormal cell proliferation disorders such as cancer, and infectious diseases. Immunostimulation in a subject can be used to achieve and/or enhance the efficacy of immune based therapies such as passive (i.e. immunoglobulin) immunotherapy, or active immunization with antigens.

The agents of the invention can be used in any therapeutic and/or prophylactic method described in U.S. Patent Nos. 4935493, 5462928, 5965532, 6040145, 6100234, 6875737, 6258597, 6300314, 6355614, and 6890904.

The agents of the invention are able to inhibit post proline-cleaving enzymes. Post proline-cleaving enzymes are enzymes which have a specificity for cleaving Xaa-Pro or Xaa-AIa dipeptides (where Xaa represents any amino acid) at the carboxy end of the dipeptide. These enzymes include dipeptidases such as DPP-IV (CD26), and endopeptidases such as prolyl endopeptidase (PEP or POP). Fibroblast activation protein (FAP) has both exo- and endo-peptidase activity. As used herein, the agents of the invention are the agents of Formulae I-XII (a-e for each).

Some agents of the invention share the common structure of **Formula Ia**



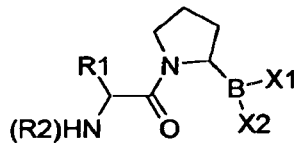
wherein T is a reactive group such as an organo boronate, an organo phosphonate, a fluoroalkylketone, an halomethyl ketone, a diazomethyl ketone, a dimethylsulphonium salt, an alphaketo carboxylic acid, an alphaketo ester, an alphaketo amide, an alpha-diketone, an acyloxymethyl ketone, an aldehyde, an epoxysuccinyl, an N-peptidyl-O-acylhydroxylamine, an azapeptide, a fluoroolefin, a peptidyl (alpha-aminoalkyl) phosphonate ester, or a nitrile.

R1 in its broadest sense may be a hydrophobic or branched side chain of an amino

acid or amino acid analog (e.g., a non-naturally occurring amino acid), or it may be an amino acid or amino acid analog (e.g., a non-naturally occurring amino acid) that comprises at least 3 carbon atoms. In one embodiment, R1 is an alkyl side chain, preferably with a branch, and more preferably with a branch at the first carbon of the side chain, and most preferably where that branch comprises a cyclic structure. It is to be understood that R1 may be comprised of carbon, oxygen, hydrogen, nitrogen, sulfur, and still other atoms. Non-carbon, non-hydrogen atoms are referred to herein as heteroatoms. In certain embodiments, R1 comprises one or more ring structures or — R3, wherein R3 comprises one or more ring structures, and R2 is a hydrogen, peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. In some embodiments, R1 comprises a five carbon ring structure. In some embodiments R3 comprises a three carbon ring structure.

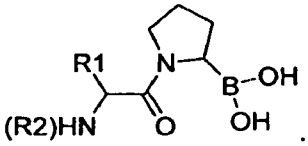
The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. The amino terminal amino acid residue may be in the L or D configuration, although in some embodiments, it is preferably in the L configuration. The proline residue attached to the reactive group is referred to as a pyrrolidine ring. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine. In some embodiments, the dipeptide moiety can be an isostere.

The agents of Formula Ia include agents of **Formula** Ib

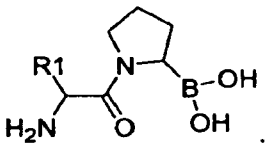


wherein X1 and X2 are independently selected from hydroxy! groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

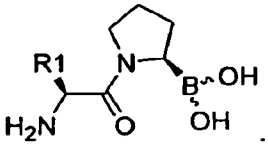
The agents of Formula Ib further include agents of **Formula Ic**



The agents of Formula Ic even further include agents of **Formula Id**

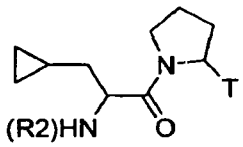


The agents of Formula Id even further include agents of **Formula Ie**



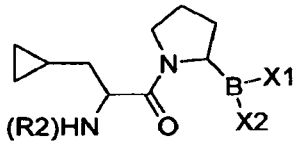
It is to be understood that the invention embraces variants in which the amino terminal side chain (e.g., R1 in the structure above) is in the R or S (or L or D) configuration, and the invention is no limited solely to the stereoisomer configurations of the "e" structures provided herein.

Some agents of the invention have the structure of **Formula Ha**



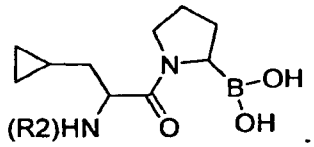
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula Ha include agents of **Formula Hb**

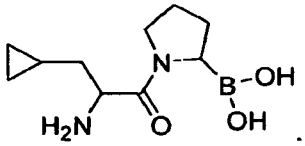


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

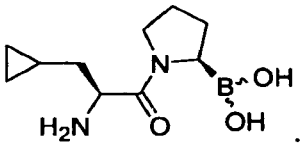
The agents of Formula lib further include agents of **Formula Hc**



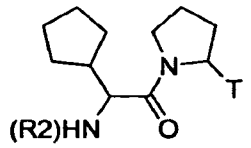
The agents of Formula lie even further include agents of **Formula Hd**



The agents of Formula Hd even further include agents of **Formula He**

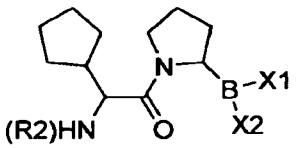


Some agents of the invention have the structure of **Formula HIa**



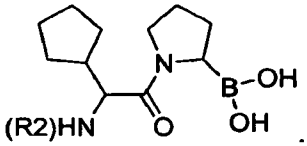
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. In some embodiments, the dipeptide moiety can be an isostere. hn some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula Ilia include agents of **Formula HIb**

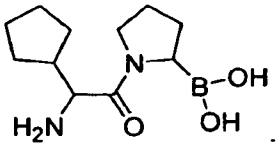


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

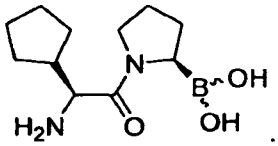
The agents of Formula IIIb further include agents of **Formula IHc**



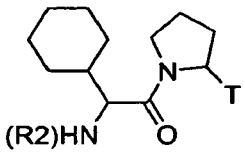
The agents of Formula IIIc even further include agents of **Formula HId**



The agents of Formula IIId even further include agents of **Formula IHe**

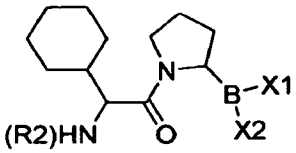


Some agents of the invention have the structure of **Formula IVa**



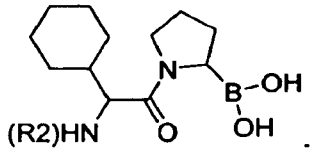
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula FVa include agents of **Formula IVb**

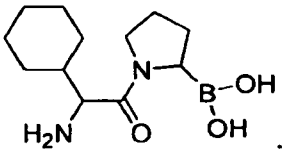


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

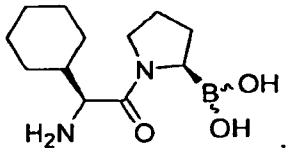
The agents of Formula IVb further include agents of **Formula IVc**



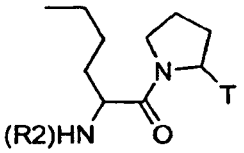
The agents of Formula IVc even further include agents of **Formula IVd**



The agents of Formula IVd even further include agents of **Formula IVe**

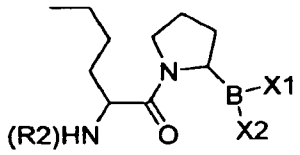


Still other agents of the invention have the structure of **Formula Va**



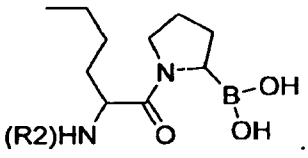
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. The amino terminal amino acid residue may be in the L or D configuration, although in some embodiments, it is preferably in the L configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula Va include agents of **Formula Vb**

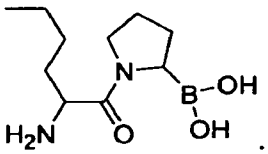


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

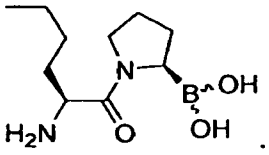
The agents of Formula Vb further include agents of **Formula Vc**



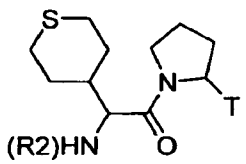
The agents of Formula Vc even further include agents of **Formula Vd**



The agents of Formula Vd even further include agents of **Formula** Ve



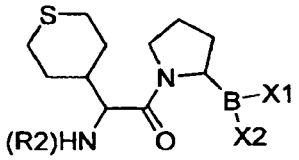
Other agents of the invention have the structure of **Formula Via**



wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in

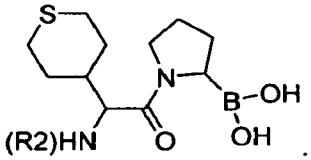
the pyrolidine ring and T may be in the D or L configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula Via include agents of **Formula VIb**

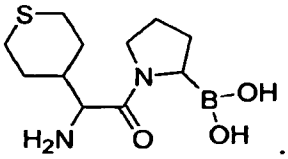


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

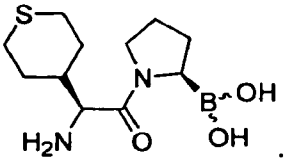
The agents of Formula VIb further include agents of **Formula VIc**



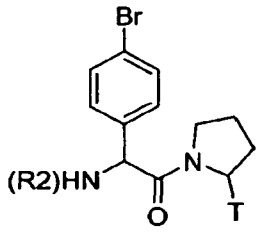
The agents of Formula VIc even further include agents of **Formula VId**



The agents of Formula VId even further include agents of **Formula VIe**

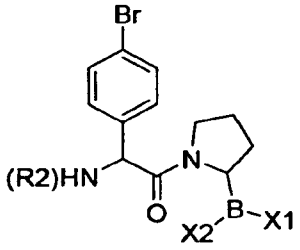


Other agents of the invention have the structure of Formula Vila



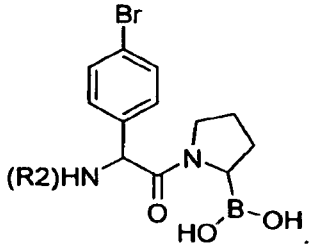
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula Vila include agents of Formula VIIb

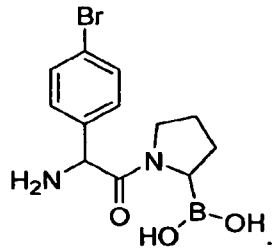


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

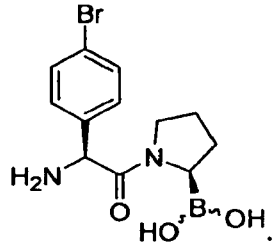
The agents of Formula VIIb further include agents of Formula VIIc



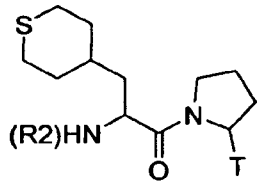
The agents of Formula VIIc even further include agents of Formula VTId



The agents of Formula VIId even further include agents of **Formula VIIe**

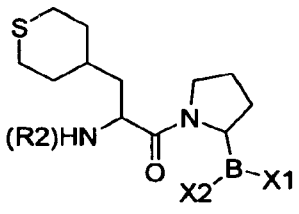


Other agents of the invention have the structure of **Formula Villa**



wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

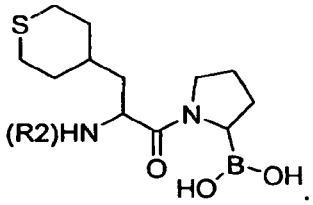
The agents of Formula Villa include agents of **Formula VIIIb**



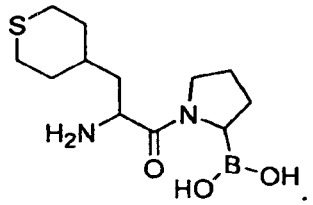
wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH.

The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

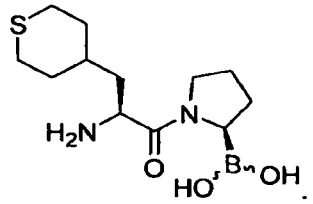
The agents of Formula VIIIb further include agents of **Formula VIIIc**



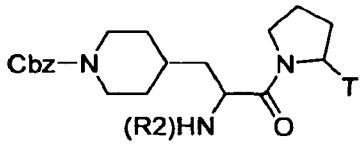
The agents of Formula VIIIc even further include agents of **Formula VIIId**



The agents of Formula VIIId even further include agents of **Formula VIIIe**

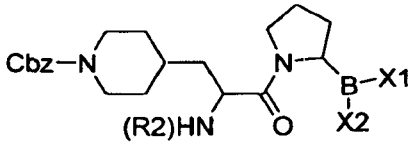


Other agents of the invention have the structure of **Formula IXa**



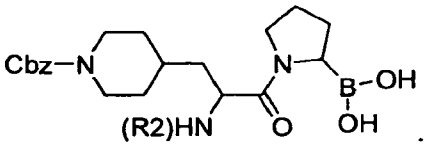
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula IXa include agents of **Formula IXb**

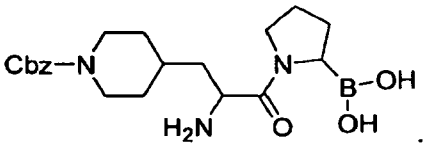


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

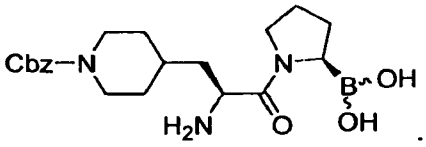
The agents of Formula IXb further include agents of **Formula IXc**



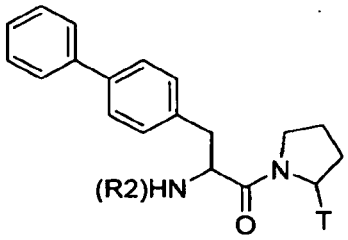
The agents of Formula IXc even further include agents of **Formula IXd**



The agents of Formula IXd even further include agents of **Formula IXe**



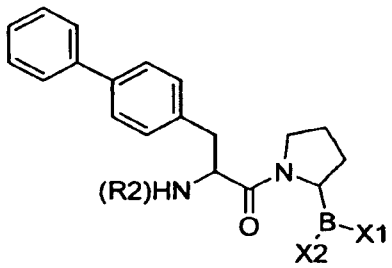
Other agents of the invention have the structure of **Formula Xa**



wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. In some embodiments, the

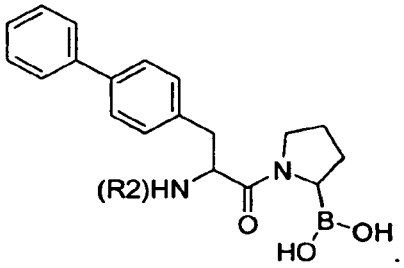
dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula Xa include agents of **Formula Xb**

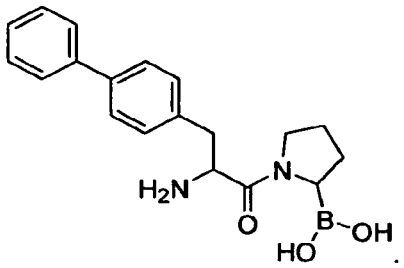


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

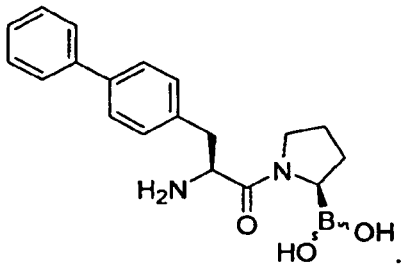
The agents of Formula Xb further include agents of **Formula Xc**



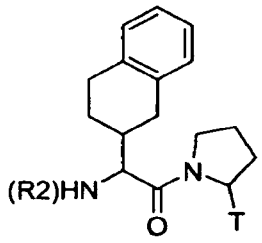
The agents of Formula Xc even further include agents of **Formula Xd**



The agents of Formula Xd even further include agents of **Formula** Xe

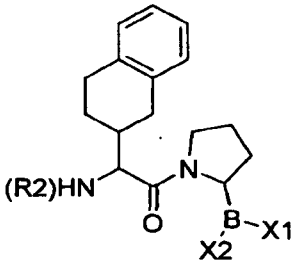


Other agents of the invention have the structure of **Formula XIa**



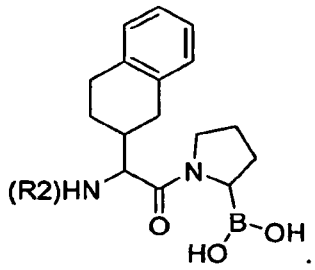
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. hi other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula XIa include agents of **Formula XIb**

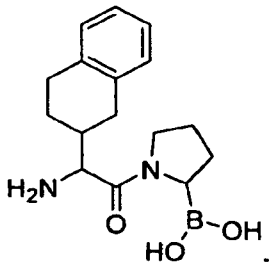


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

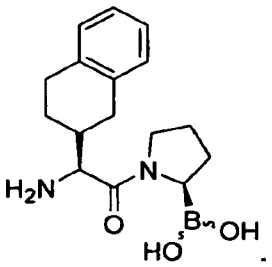
The agents of Formula XIb further include agents of **Formula XIc**



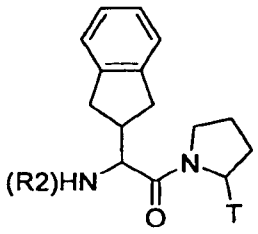
The agents of Formula XIc even further include agents of **Formula XId**



The agents of Formula XId even further include agents of **Formula XIe**



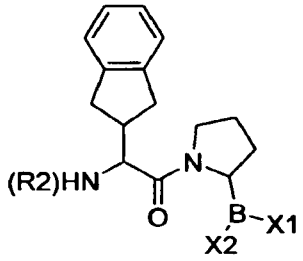
Other agents of the invention have the structure of **Formula XIIa**



wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced

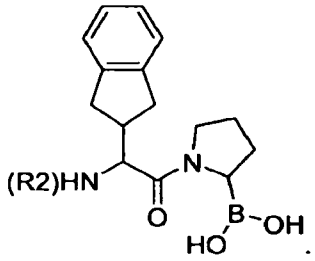
with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula XIIa include agents of **Formula XIIb**

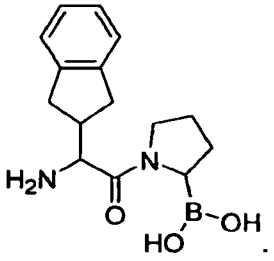


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

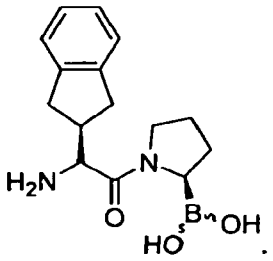
The agents of Formula XIIb further include agents of **Formula XIIc**



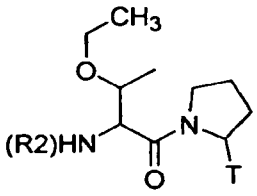
The agents of Formula XIIc even further include agents of **Formula XIId**



The agents of Formula XIId even further include agents of Formula **XIIe**

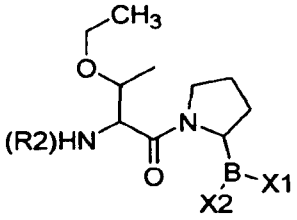


Other agents of the invention have the structure of **Formula XIIIa**



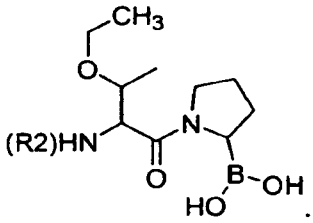
wherein T is a reactive group as described above; and R2 is a peptide, peptidomimetic, or amino acid, whether naturally or non-naturally occurring. The bond between the carbon in the pyrolidine ring and T may be in the D or L configuration. The amino terminal amino acid residue may be in the L or D configuration, although in some embodiments, it is preferably in the L configuration. In some embodiments, the dipeptide moiety can be an isostere. In some embodiments, the pyrrolidine may be replaced with an azetidine or a thiazolidine. In other embodiments, the pyrrolidine-T moiety is replaced with a 4-cyanothiazolidine.

The agents of Formula XIIIa include agents of **Formula XIIIb**

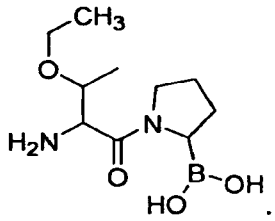


wherein X1 and X2 are independently selected from hydroxyl groups or groups capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. The bond between the C and B is preferably in the L configuration (otherwise referred to as the R configuration).

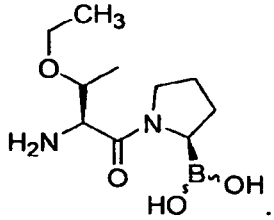
The agents of Formula XIIIb further include agents of **Formula XIIIc**



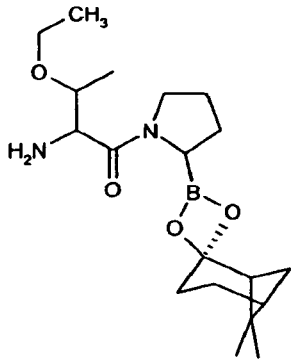
The agents of Formula XIIIc even further include agents of **Formula XIIId**



The agents of Formula XIIId even further include agents of **Formula XIIIe**

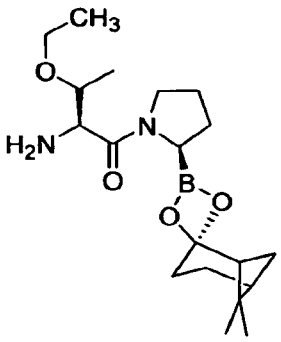


An example of an agent that converts to an agent of Formulae XIIId when exposed to aqueous solution is represented by **Formula XIIIg**



The agent of Formula XIIIg contains the pinanediol protective group which is present during synthesis of the agent. This group spontaneously hydrolyzes to hydroxyls in the presence of water.

An example of an agent that converts to an agent of Formulae XIIIe when exposed to aqueous solution is represented by **Formula XIIIf**



R2 can be composed of hydrogen, or single or multiple amino acid residues, whether naturally occurring or not, or a peptide or a peptidomimetic. The residues may be attached to each other via peptide bonds or non-peptide bonds (such as in an isostere). Therefore R2 can be composed wholly of amino acid residues, wholly of non-amino acid substituents, or a combination of both. R2 may be 100 or more residues in length including 30, 20, 10 or less than 10 residues in length. R2 or a portion(s) thereof may mimic a substrate of the protease.

R2 can be synthesized from other biomolecules including but not limited to saccharides, fatty acids, sterols, isoprenoids, purines, pyrimidines, derivatives or structural analogs of the above, or combinations thereof and the like. Also envisioned in the invention is the use of targeting groups made from peptoids, random bio-oligomers (U.S. Patent 5,650,489), benzodiazepines, diversomeres such as dydantoins, benzodiazepines and dipeptides, nonpeptidyl peptidomimetics with a beta-D-glucose scaffolding, oligocarbamates or peptidyl phosphonates.

Many if not all of these agents can be synthesized using recombinant or chemical library approaches. A vast array of candidate R2 groups can be generated from libraries of synthetic or natural compounds. The methods of the invention utilize this library technology to identify small molecules which bind to protease reactive sites. One advantage of using libraries for inhibitor identification is the facile manipulation of millions of different putative candidates of small size in small reaction volumes (i.e., in synthesis and screening reactions). Another advantage of libraries is the ability to synthesize targeting groups which might not otherwise be attainable using naturally occurring sources.

The use of library technology, such as phage display, and combinatorial chemistry, such as compound array methods, in the synthesis and screening of protease inhibitors has been previously described in U.S. Patent No. 6875737 entitled "Multivalent Compounds for

Crosslinking Receptors and Uses Thereof" issued on April 5, 2005, the contents of which are incorporated in their entirety by reference. Examples of parallel synthesis mixtures and parallel synthesis methods are provided in PCT published patent application W095/18972, published July 13, 1995 and U.S. Patent No. 5,712,171 granted January 27, 1998 and its corresponding PCT published patent application W096/22529, which are hereby incorporated by reference.

Libraries can be screened to identify naturally or non-naturally occurring putative R2 groups by assaying protease binding (and optionally cleavage activity) in the presence of the library molecule or member. The cleavage assay includes determining whether the library molecule inhibits cleavage by the protease of a known substrate or of a substrate analog (e.g., a chromophoric substrate analog which is easily detectable in a spectrophotometric assay). Those library molecules which exhibit binding and optionally inhibition of a post-prolyl cleaving enzyme then can be covalently coupled to the reactive groups disclosed herein and again tested for binding and inhibition of the enzyme. In this manner, a simple, high-throughput screening assay is provided for identifying inhibitors.

It is to be understood that each and every reactive group described herein can be used in any of the foregoing structural formulae. Thus, other inhibitors have an analogous structure but with the boronyl group replaced by, for example, an organo phosphonate, a fluoroalkylketone, an halomethyl ketone, a diazomethyl ketone, a dimethylsulphonium salt, an alphaketo carboxylic acid, an alphaketo ester, an alphaketo amide, an alpha-diketone, an acyloxymethyl ketone, an aldehyde, an epoxysuccinyl, an N-peptidyl-O-acylhydroxylamine, an azapeptide, a fluoroolefin, a peptidyl (alpha-aminoalkyl) phosphonate ester, or a nitrile.

All amino acids, with the exception of glycine, contain an asymmetric or chiral carbon and may contain more than one chiral carbon atom. The asymmetric α carbon atom of the amino acid is referred to as a chiral center and can occur in two different isomeric forms. These forms are identical in all chemical and physical properties with one exception, the direction in which they can cause the rotation of plane-polarized light. These amino acids are referred to as being "optically active," i.e., the amino acids can rotate the plane-polarized light in one direction or the other.

The four different substituent groups attached to the α carbon can occupy two different arrangements in space. These arrangements are not superimposable mirror images of each other and are referred to as optical isomers, enantiomers, or stereo isomers. A solution of one

stereo isomer of a given amino acid will rotate plane polarized light to the left and is called the levorotatory isomer [designated (-)]; the other stereo isomer for the amino acid will rotate plane polarized light to the same extent but to the right and is called dextrorotatory isomer [designated (+)].

A more systematic method for classifying and naming stereo isomers is the absolute configuration of the four different substituents in the tetrahedron around the asymmetric carbon atom (e.g., the α carbon atom). To establish this system, a reference compound was selected (glyceraldehyde), which is the smallest sugar to have an asymmetric carbon atom. By convention in the art, the two stereo isomers of glyceraldehyde are designated L and D. Their absolute configurations have been established by x-ray analysis. The designations, L and D, also have been assigned to the amino acids by reference to the absolute configuration of glyceraldehyde. Thus, the stereo isomers of chiral compounds having a configuration related to that of L-glyceraldehyde are designed L, and the stereo isomers having a configuration related to D-glyceraldehyde are designated D, regardless of the direction in which they rotate the plane-polarized light. Thus, the symbols, L and D, refer to the absolute configuration of the four substituents around the chiral carbon.

In general, naturally occurring compounds which contain a chiral center are only in one stereo isomeric form, either D or L. The naturally occurring amino acids are the L stereo isomers. As discussed herein, the invention embraces inhibitors comprising amino acid residues which can be in the D stereo isomer configuration also.

Most amino acids that are found in proteins can be unambiguously named using the D L system. However, compounds which have two or more chiral centers may be in 2^n possible stereo isomer configurations, where n is the number of chiral centers. These stereo isomers sometimes are designated using the RS system to more clearly specify the configurations of amino acids that contain two or more chiral centers. For example, compounds such as threonine and isoleucine contain two asymmetric carbon atoms and therefore have four stereo isomer configurations. The isomers of compounds having two chiral centers are known as diastereomers. A complete discussion of the RS system of designating optical isomers for amino acids is provided in Principles in Biochemistry, editor A.L. Lehninger, page 99-100, supra. A brief summary of this system follows.

The RS system was invented to avoid ambiguities when a compound contains two or more chiral centers. In general, the system is designed to rank the four different substituent

atoms around an asymmetric carbon atom in order of decreasing atomic number or in order of decreasing valance density when the smallest or lowest-rank group is pointing directly away from the viewer. The different rankings are well known in the art and are described on page 99 of Lehninger (supra). If the decreasing rank order is seen to be clock-wise, the configuration around the chiral center is referred to as R; if the decreasing rank order is counter-clockwise, the configuration is referred to as S. Each chiral center is named accordingly using this system. Applying this system to threonine, one skilled in the art would determine that the designation, L-threonine, refers to (2S, 3R)-threonine in the RS system. The more traditional designations of L-, D-, L-allo, and D-allo, for threonine have been in common use for some time and continue to be used by those of skill in this art. However, the R S system increasingly is used to designate the amino acids, particularly those which contain more than one chiral center.

Thus, the agents may comprise the carbon of the pyrrolidine ring bonded to a boron (B) in the R-configuration. The agent may be provided as a mixture of R- and S-enantiomers of boron substituted pyrrolidine. In a related embodiment the mixture of R- and S-enantiomers of boron substituted pyrrolidine contains at least 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% of the R-enantiomer of boron substituted pyrrolidine. Similarly, at least 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% of the carbon atoms bearing boron are of the L-configuration. Such agents are referred to as being optically pure. Methods for synthesizing optically pure isomers are disclosed in published PCT application WO 93/08259. The C to Rl bond may be in the L or D configuration, although in some embodiments, it is preferably in the L configuration. Similarly, the amino terminal amino acid is structures such as I-XIII may be in the L or D configuration, although it is preferred that they be in the L configuration in some embodiments.

The agents of the invention may be provided as agents that are converted *(via* enzymatic, chemical, metabolic, or any other means, *in vivo* or *ex vivo)* to the structures shown herein. These parent agents include prodrugs. A prodrug, as used herein, is an agent that is metabolized *in vivo* to yield another agent or that disintegrates (e.g., upon contact with stomach acid) to form the other agent. Some prodrugs are converted via hydrolysis or oxidation *in vivo.* These include alcohol precursors of that are oxidized *in vivo* (e.g., in the liver) and a boroxine derivatives, as well as esters and related compounds. Prodrugs also include cyclized versions of the molecule, as discussed above.

Another category of prodrugs includes compounds that are converted by enzymes. These enzymes may be post-prolyl cleaving enzymes (e.g., DPP-IV) or non-post-prolyl cleaving enzymes. Examples of this class of prodrug moieties are disclosed in U.S. Patent Nos. 5,462,928 issued October 31, 1995; and 6,100,234 issued August 8, 2000; and published PCT applications WO 91/16339 published October 31, 1991; WO 93/08259 published April 29, 1993; and WO 03/092605, published November 13, 2003, among others.

The length of prodrugs may be 4, 6, 8, 10, 12, 14, 16, 18, 20, 30, 50, 100 or more residues in length. Multiples of 3 are also contemplated. The residues may be naturally or non-naturally occurring amino acids, as described herein.

In some embodiments, the prodrug has an R2 group having one or more dipeptides or tripeptides, optionally having a carboxy terminal praline residue. Such dipeptides and tripeptides may be repeated once, twice or more (e.g., 5 times, 10 times or more). As an example, prodrugs of Formula V include but are not limited to Ala-Pro-NLeu-L-boroPro, Asp-Pro-NLeu-L-boroPro, Glu-Pro-NLeu-L-boroPro, Asn-Pro-NLeu-L-boroPro, Gln-Pro-NLeu-L-boroPro, Lys-Pro-NLeu-L-boroPro, Arg-Pro-NLeu-L-boroPro, His-Pro-NLeu-L-boroPro, Pro-Pro-NLeu-L-boroPro, Thr-Pro-NLeu-L-boroPro, Ser-Pro-NLeu-L-boroPro, Cys-Pro-NLeu-L-boroPro, Gly-Pro-NLeu-L-boroPro, Tyr-Pro-NLeu-L-boroPro, Trp-Pro-NLeu-L-boroPro, Phe-Pro-NLeu-L-boroPro, Leu-Pro-NLeu-L-boroPro, Ile-Pro-NLeu-L-boroPro, Met-Pro-NLeu-L-boroPro, or Val-Pro-NLeu-L-boroPro.

These agents can be provided in linear or cyclic form or as mixtures thereof, as described in U.S. Patent No. 6,355,614, issued March 12, 2002. The percentage of cyclic forms (relative to linear forms) in these mixtures may vary (e.g., less than 20% to more than 90% including at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more cyclic) depending on the formulation.

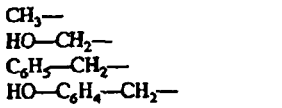
The amino acid residues may be naturally and non-naturally occurring amino acids. Examples of naturally occurring amino acids are glycine (GIy), and the L-forms of alanine (Ala), valine (VaI), leucine (Leu), isoleucine (He), phenylalanine (Phe), tyrosine (Tyr), tryptophan (Trp), cysteine (Cys), methionine (Met), serine (Ser), threonine (Thr), lysine (Lys), arginine (Arg), histidine (His), aspartic acid (Asp), glutamic acid (GIu), asparagine (Asn), glutamine (GIn) and proline (Pro).

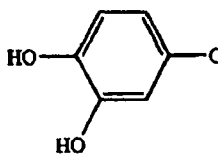
Non-naturally occurring amino acids include the D-forms of Ala, VaI, Leu, He, Phe, Tyr, Trp, Cys, Met, Ser, Thr, Lys, Arg, His, Asp, GIu, Asn, GIn, and Pro.

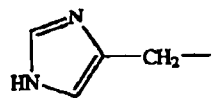
Other examples of non-naturally occurring amino acids include 2-azetidinecarboxylic acid or pipecolic acid (which have 6-membered, and 4-membered ring structures respectively), 4-hydroxy-proline (Hyp), 5-hydroxy-lysine, norleucine (NIe), 5-hydroxynorleucine (Hyn), 6-hydroxynorleucine, ornithine, cyclohexylglycine (Chg), N-Methylglycine (N-MeGIy), N-Methylalanine (N-MeAIa), N-Methylvaline (N-MeVaI), N-Methylleucine (N-MeLeu), N-Methylisoleucine (N-MeIIe), N-Methylnorleucine (N-MeNIe), N-Methyl-2-aminobutyric acid (N-MeAbu) and N-Methyl-2-aminopentanoic acid (N-MeNva), methylthreonine, nitroglutamine, norleucine (NIe), norvaline, ornithine, phosphoserine, pipecolic acid, sarcosine, taurine, tert-leucine, thiazolidine carboxylic acid, thyroxine, trans-4-hydroxyproline, and trans-3-methylproline.

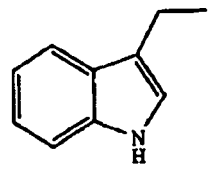
Non-naturally occurring amino acids also include beta-amino acids and alpha-amino acids with side chains replaced with synthetic derivatives. Representative side chains of naturally occurring and non-naturally occurring α-amino acids are shown below in Table 1.

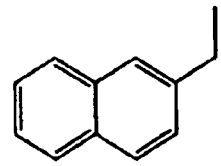
Table 1

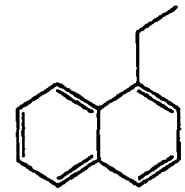


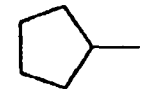












Non-naturally occurring amino acids also include D, L, and racemic configurations of hydrophobic amino acids. Hydrophobic amino acids include amino acid analogs having the formula -NH-CHR-CO-, wherein R is an aliphatic group, a substituted aliphatic group, a benzyl group, a substituted benzyl group, an aromatic group or a substituted aromatic group and wherein R does not correspond to the side chain of a naturally-occurring amino acid. As used herein, aliphatic groups include straight chained, branched or cyclic C1-C8 hydrocarbons which are completely saturated, which contain one or two heteroatoms such as nitrogen, oxygen or sulfur and/or which contain one or more units of desaturation. Aromatic groups include carbocyclic aromatic groups such as phenyl and naphthyl and heterocyclic aromatic groups such as imidazolyl, indolyl, thienyl, furanyl, pyridyl, pyranyl, oxazolyl, benzothienyl, benzofuranyl, quinolinyl, isoquinolinyl and acridintyl.

Suitable substituents on an aliphatic, aromatic or benzyl group include -OH, halogen (-Br, -Cl, -I and -F) -O (aliphatic, substituted aliphatic, benzyl, substituted benzyl, aryl or substituted aryl group), -CN, $-NO_2$, -COOH, $-NH_2$, -NH (aliphatic group, substituted aliphatic, benzyl, substituted benzyl, aryl or substituted aryl group), -N (aliphatic group, substituted aliphatic, benzyl, substituted benzyl, aryl or substituted aryl group)$_2$, -COO (aliphatic group, substituted aliphatic, benzyl, substituted benzyl, aryl or substituted aryl group), $-CONH_2$, -CONH (aliphatic, substituted aliphatic group, benzyl, substituted benzyl, aryl or substituted aryl group)), -SH, -S (aliphatic, substituted aliphatic, benzyl, substituted benzyl, aromatic or substituted aromatic group) and -NH-C(=NH)-NH2. A substituted benzylic or aromatic group can also have an aliphatic or substituted aliphatic group as a substituent. A substituted aliphatic group can also have a benzyl, substituted benzyl, aryl or substituted aryl group as a substituent. A substituted aliphatic, substituted aromatic or substituted benzyl group can have one or more substituents. Modifying an amino acid substituent can increase, for example, the lypophilicity or hydrophobicity of natural amino acids which are hydrophilic.

A number of the suitable amino acids, amino acids analogs and salts thereof can be obtained commercially. Others can be synthesized by methods known in the art. Synthetic techniques are described, for example, in Green and Wuts, *"Protecting Groups in Organic Synthesis"*, John Wiley and Sons, Chapters 5 and 7, 1991.

It should be understood that reference to any particular Formula herein is intended solely for convenience and such reference applies equally to Formulae I-XIII (and a-e versions thereof), unless otherwise noted.

The agents of the invention are provided in some instances in isolated form. As used herein, isolated means that the agents are separated from synthesis reagents, catalysts, enzymes, and byproducts. An isolated agent may represent at least 50%, 60%, 70%, 80%, 90%, 95%, 99%, or 100% by weight of the composition in which they exist.

The agents may be used alone or in combination with other therapeutic agents (referred to herein as second therapeutic agents). Second therapeutic agents include but are not limited to anti-cancer agents, anti-microbial agents, antigens, antibodies and antigen-binding fragments thereof, adjuvants, chemokines, cytokines, and the like.

Several aspects of the invention exploit the immunostimulatory activity of agents of the invention in compositions or methods that use these agents alone or in combination with secondary agents such as but not limited to antibodies (or antigen-binding fragments thereof) and antigens. The immune response can be an innate immune response (i.e., immunity mediated by neutrophils, macrophages, NK cells and eosinophils) or an adaptive (antigen-specific) immune response (i.e., immunity mediated by T cells and B cells).

The novel observation that agents of the invention induce the production of cytokines such as IL-1β and G-CSF and CXCL chemokines such as CXCL1/KC and CXCL8/IL-8 indicates that they can be used for a number of indications and conditions that are mediated fully or in part by these cytokines and chemokines and their downstream signaling events. Some of these indications are recited herein as targets of monotherapy or combination therapy using agents of the invention. For instance, the ability of the agents to induce IL-1β suggests that these agents are useful in vaccine induced immunity, including both humoral and cell-mediated immunity. The ability of the agents to cause the activation of caspase-1 (also called IL-1β converting enzyme or ICE) in cells of the macrophage-monocyte lineage suggests that their primary biological activity is the induction of IL-Iβ by the conversion of intracellular pro-IL-1β into the mature and biologically active form of IL-Iβ that is secreted. The ability to enhance cellular mediated immunity is useful, inter alia, in the treatment or prevention of viral infections, and in particular, HIV infection. Thus, as described below, agents of the invention may be used together with vaccines such as those to small pox virus (e.g., BVL).

The compositions provided herewith may also be used, as described below, for a variety of mucosal immunities. Mucosal immunity generally involves immunoglobulin of the

secretory IgA (s-IgA) isotype, and accordingly, antibodies of this isotype could be used together with the agents of the invention, although such antibodies are not so limited. The agents of the invention are useful in stimulating both cell-mediated immune responses and antibody-mediated immune responses at mucosal surfaces. Mucosal surfaces include nasal, oral, rectal, vaginal and gastrointestinal surfaces. In some of these methods, the agent of the invention is administered to the mucosal surface in an enterically coated form. The other therapeutic agents which work in concert with agents of the invention can be administered directly to a mucosal surface, although this is not required.

The agents of the invention may be used to enhance immunity in a subject at risk of developing a condition that is immunologically responsive. For example, a subject may be administered an agent of the invention when it is at risk of developing the flu. As another example, a subject having or at risk of having angina may be administered an agent of the invention.

The agents of the invention may be used to treat disorders characterized by abnormal cell proliferation. An abnormal cell proliferation disorder or condition, as used herein, refers to a localized region of cells which may exhibit an abnormal (e.g., increased) rate of division as compared to their normal tissue counterparts or an abnormal response to growth or inhibitory signals. These conditions include but are not limited to conditions involving solid tumor masses of benign, pre-malignant or malignant character. These conditions also include the cancers recited herein.

Thus, the agents of the invention may be administered to subjects having or at risk of developing cancer. A subject having a cancer is a subject that has detectable cancerous cells. A subject at risk of developing a cancer is one who has a higher than normal probability of developing cancer. These subjects include, for instance, subjects having a genetic abnormality that has been demonstrated to be associated with a higher likelihood of developing a cancer, subjects having a familial disposition to cancer, subjects exposed to cancer causing agents (i.e., carcinogens) such as tobacco, asbestos, or other chemical toxins, and subjects previously treated for cancer and in apparent remission.

In some important embodiments of the invention, the methods are particularly directed to subjects at high risk of cancer, such as those predisposed for familial (e.g., familial colon polyposis, BRCAl- or BRCA2- associated breast cancer, Wilms tumour, colorectal cancer, Li-Fraumeni Syndrome, ovarian cancer, and prostate cancer) or non-familial genetic reasons. Subjects at high risk are also those that manifest pre-cancerous symptoms such as pre-

cancerous polyps (e.g., in colon cancer), or pre-cancerous lesions (e.g., in HPV-induced cervical cancer).

"Cancer" as used herein refers to an uncontrolled growth of cells which interferes with the normal functioning of the bodily organs and systems. Hemopoietic cancers, such as leukemia, are able to outcompete the normal hemopoietic compartments in a subject, thereby leading to hemopoietic failure (in the form of anemia, thrombocytopenia and neutropenia) ultimately causing death.

A cancer cell is a cell that divides and reproduces abnormally due to a loss of normal growth control. Cancer cells almost always arise from at least one genetic mutation. In some instances, it is possible to distinguish cancer cells from their normal counterparts based on profiles of expressed genes and proteins, as well as to the level of their expression. Genes commonly affected in cancer cells include oncogenes, such as ras, neu/HER2/erbB, myb, myc and abl, as well as tumor suppressor genes such as p53, Rb, DCC, RET and WT. Cancer-related mutations in some of these genes leads to a decrease in their expression or a complete deletion. In others, mutations cause an increase in expression or the expression of an activated variant of the normal counterpart.

The term "tumor" is usually equated with neoplasm, which literally means "new growth" and is used interchangeably with "cancer." A "neoplastic disorder" is any disorder associated with cell proliferation, specifically with a neoplasm. A "neoplasm" is an abnormal mass of tissue that persists and proliferates after withdrawal of the carcinogenic factor that initiated its appearance. There are two types of neoplasms, benign and malignant. Nearly all benign tumors are encapsulated and are noninvasive; in contrast, malignant tumors are almost never encapsulated but invade adjacent tissue by infiltrative destructive growth. This infiltrative growth can be followed by tumor cells implanting at sites discontinuous with the original tumor. The method of the invention can be used to treat neoplastic disorders in humans, including but not limited to: sarcoma, carcinoma, fibroma, leukemia, lymphoma, melanoma, myeloma, neuroblastoma, rhabdomyosarcoma, retinoblastoma, and glioma as well as each of the other tumors described herein.

Cancers that migrate from their original location and seed vital organs (thereby giving rise to metastatic lesions) can eventually lead to the death of the subject through the functional deterioration of the affected organs. A metastasis is a region of cancer cells, distinct from the primary tumor location resulting from the dissemination of cancer cells from the primary tumor to other parts of the body. Thus, subjects with metastatic tumors can also be treated

according to the invention. In some embodiments, the metastatic tumors are of epithelial origin. Carcinomas may metastasize to bone, as has been observed, for example, with breast cancer, and liver cancer, as sometimes with colon cancer. The methods of the invention are intended to treat metastatic tumors regardless of the site of the metastasis and/or the site of the primary tumor. In preferred embodiments, the metastases are of epithelial origin.

Cancers include, but are not limited to, basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and CNS cancer; breast cancer; cervical cancer; choriocarcinoma; colon and rectum (i.e., colorectal) cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer; germ cell tumors; intra-epithelial neoplasm; Kaposi's sarcoma; kidney cancer; larynx cancer; leukemia (e.g., acute myeloid leukemia, acute lymphoid leukemia, chronic myeloid leukemia and chronic lymphoid leukemia); liver cancer; lung cancer (e.g. small cell lung cancer and non-small cell lung cancer); lymphoma including Hodgkin's andNon-Hodgkin's lymphoma; melanoma; myeloma; neuroblastoma; oral cavity cancer (e.g., lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; renal cell cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; renal cancer; cancer of the respiratory system; sarcoma; skin cancer; stomach cancer; stromal tumors; testicular cancer; thyroid cancer; uterine cancer; cancer of the urinary system, as well as other carcinomas and sarcomas.

Carcinomas are cancers of epithelial origin that include, but are not limited to, acinar carcinoma, acinous carcinoma, alveolar adenocarcinoma (also called adenocystic carcinoma, adenomyoepithelioma, cribriform carcinoma and cylindroma), carcinoma adenomatosum, adenocarcinoma, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma (also called bronchiolar carcinoma, alveolar cell tumor and pulmonary adenomatosis), basal cell carcinoma, carcinoma basocellulare (also called basaloma, or basiloma, and hair matrix carcinoma), basaloid carcinoma, basosquamous cell carcinoma, breast carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma (also called cholangioma and cholangiocarcinoma), chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epibulbar carcinoma, epidermoid carcinoma, carcinoma epitheliale adenoides, carcinoma exulcere, carcinoma fibrosum, gelatiniform carcinoma, gelatinous carcinoma, giant

cell carcinoma, gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma (also called hepatoma, malignant hepatoma and hepatocarcinoma), Hürthle cell carcinoma, hyaline carcinoma, hypernephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma mastitoides, carcinoma medullare, medullary carcinoma, carcinoma melanodes, melanotic carcinoma, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, nasopharyngeal carcinoma, carcinoma nigrum, non-small-cell lung carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, ovarian carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prostate carcinoma, renal cell carcinoma of kidney (also called adenocarcinoma of kidney and hypernephoroid carcinoma), reserve cell carcinoma, carcinoma sarcomatodes, scheinderian carcinoma, scirrhous carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell lung carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, carcinoma vilosum. In preferred embodiments, the methods of the invention are used to treat subjects having cancer of the breast, cervix, ovary, prostate, lung, colon and rectum, pancreas, stomach or kidney.

Another particularly important cancer type is sarcomas. Sarcomas are rare mesenchymal neoplasms that arise in bone and soft tissues. Different types of sarcomas are recognized and these include: liposarcomas (including myxoid liposarcomas and pleomorphic liposarcomas), leiomyosarcomas, rhabdomyosarcomas, malignant peripheral nerve sheath tumors (also called malignant schwannomas, neurofibrosarcomas, or neurogenic sarcomas), Ewing's tumors (including Ewing's sarcoma of bone, extraskeletal (i.e., non-bone) Ewing's sarcoma, and primitive neuroectodermal tumor [PNET]), synovial sarcoma, angiosarcomas, hemangiosarcomas, lymphangiosarcomas, Kaposi's sarcoma, hemangioendothelioma, fibrosarcoma, desmoid tumor (also called aggressive fibromatosis), dermatofibrosarcoma protuberans (DFSP), malignant fibrous histiocytoma (MFH), hemangiopericytoma, malignant mesenchymoma, alveolar soft-part sarcoma, epithelioid sarcoma, clear cell sarcoma, desmoplastic small cell tumor, gastrointestinal stromal tumor

(GIST) (also known as GI stromal sarcoma), osteosarcoma (also known as osteogenic sarcoma)-skeletal and extraskeletal, and chondrosarcoma.

The methods of the invention are also directed towards the treatment of subjects with melanoma. Melanomas are tumors arising from the melanocytic system of the skin and other organs. Examples of melanoma include lentigo malignant melanoma, superficial spreading melanoma, nodular melanoma, and acral lentiginous melanoma.

In one aspect, the invention involves treating conditions involving a tumor mass which contains or is dependent upon the presence of reactive stromal fibroblasts at some point during its development. As used herein, reactive fibroblasts are fibroblasts which have been activated to express proteins such as receptors and growth factors which, in some instances, have a positive effect and, in other instances, have a negative effect on cellular proliferation and growth of the fibroblasts themselves, and other cell types such as malignant cells of a carcinoma or epithelial metastasis.

The cancers to be treated may be refractory cancers. A refractory cancer as used herein is a cancer that is resistant to the ordinary standard of care prescribed. These cancers may appear initially responsive to a treatment (and then recur), or they may be completely non-responsive to the treatment. The ordinary standard of care will vary depending upon the cancer type, and the degree of progression in the subject. It may be a chemotherapy, surgery, or radiation, or a combination thereof. Those of ordinary skill in the art are aware of such standards of care. Subjects being treated according to the invention for a refractory cancer therefore may have already been exposed to another treatment for their cancer. Alternatively, if the cancer is likely to be refractory (e.g., given an analysis of the cancer cells or history of the subject), then the subject may not have already been exposed to another treatment. Examples of refractory cancers include but are not limited to leukemia, Non-Hodgkin's lymphoma, melanoma, renal cell carcinoma, colon cancer, colon cancer, liver (hepatic) cancer, pancreatic cancer, and lung cancer.

In some embodiments, a cancer that is refractory to the ordinary standard of care may be treated using a combination of one or more agents of the invention together with the ordinary standard of care. Thus, the agents of the invention may alter the responsiveness of a cancer to a particular secondary therapeutic agent.

The compositions and methods of the invention in certain instances may be useful for replacing existing surgical procedures or drug therapies, although in most instances the present invention is useful in improving the efficacy of existing therapies for treating such

conditions. Accordingly combination therapy may be used to treat the subjects that are undergoing or that will undergo a treatment for inter alia cancer or infectious disease. For example, the agents may be administered to a subject in combination with another anti¬proliferative (e.g., an anti-cancer) therapy. Suitable anti-cancer therapies include surgical procedures to remove the tumor mass, chemotherapy or localized radiation. The other anti-proliferative therapy may be administered before, concurrent with, and/or after treatment with the agent of the invention. There may also be a delay of several hours, days and in some instances weeks between the administration of the different treatments, such that the agent may be administered before or after the other treatment. In some embodiments, the agents of the invention may be administered with or without the antigens or antibodies, prior to the administration of the other antiproliferative treatment (e.g., prior to surgery, radiation or chemotherapy), although the timing is not so limited.

Although not intending to be bound by any particular mechanism, it is proposed that the administration of agents of the invention induces memory within the immune cell compartment, for example, by the induction of memory T cells, and B cells. This is believed to occur via the cytokine cocktail that is induced by agents of the invention, particularly the induction of EL-I β and the downstream activation of the immune system by the secreted mature form of IL-I β. The ability to generate memory T cells can enhance immune responses to, for example, cancerous cells that are remaining following a surgical procedure, or following chemotherapy or radiation.

The invention further contemplates the use of agents of the invention in cancer subjects prior to and following surgery, radiation or chemotherapy in order to create memory immune cells to the cancer antigen. In this way, memory cells of the immune system can be primed with cancer antigens and thereby provide immune surveillance in the long term. This is particularly suited to radiotherapy of subjects where immune cells so primed can invade a tumor site and effectively clear any remaining tumor debris. This in turn promotes further immunity to the cancer, particularly to antigens that might not have been exposed in the context of a tumor mass pre-treatment.

The invention contemplates the use of an agent of the invention with a secondary therapy such as but not limited to an anti-cancer agent, whereby the combined use of these agents enhances the effect of either when used alone. This may be the case particularly where the second agent is the ordinary standard of care in the art. The subject being treated may be one that has already been administered the ordinary standard of care and not responded well

or not responded at all to it (see below for refractory cancer discussion). The combined use of agents may make the subject responsive to the ordinary standard of care to a greater extent than in the absence of the agent of the invention.

The agents of the invention may be used together with other antiproliferative (e.g., an anti-cancer) therapies to treat subjects. The other anti-proliferative therapies that can be administered to a subject together with the agents of the invention are recited herein, and include for example, chemotherapeutic agents, antibodies and antibody fragments, and antigens.

As used herein, an anti-cancer therapy is a therapy effective in treating or preventing a cancer. Suitable anti-cancer therapies include surgical procedures to remove the tumor mass, chemotherapy or localization radiation. The other anti-proliferative therapy may be administered before, concurrent with, or after treatment with the agent of the invention. There may also be a delay of several hours, days and in some instances weeks between the administration of the different treatments, such that the agent may be administered before or after the other treatment.

As an example, the agents of the invention may be administered in combination with surgery to remove an abnormal proliferative cell mass. As used herein, "in combination with surgery" means that the agent may be administered prior to, during or after the surgical procedure. Surgical methods for treating cancer include intra-abdominal surgeries such as right or left hemicolectomy, sigmoid, subtotal or total colectomy and gastrectomy, radical or partial mastectomy, prostatectomy and hysterectomy. In these embodiments, the agent may be administered either by continuous infusion or in a single bolus. Administration during or immediately after surgery may include a lavage, soak or perfusion of the tumor excision site with a pharmaceutical preparation of the agent in a pharmaceutically acceptable carrier. In some embodiments, the agent is administered at the time of surgery as well as following surgery in order to inhibit the formation and development of metastatic lesions. The administration of the agent may continue for several hours, several days, several weeks, or in some instances, several months following a surgical procedure to remove a tumor mass.

The subjects can also be administered the agents in combination with non-surgical anti-proliferative (e.g., anti-cancer) drug therapy. Some anti-cancer agents can be categorized as DNA damaging agents and these include topoisomerase inhibitors (e.g., etoposide, ramptothecin, topotecan, teniposide, mitoxantrone), anti-microtubule agents (e.g., vincristine, vinblastine), anti-metabolic agents (e.g., cytarabine, methotrexate, hydroxyurea, 5-

fluorouracil, floxuridine, 6-thioguanine, 6-mercaptopurine, fludarabine, pentostatin, chlorodeoxyadenosine), DNA alkylating agents (e.g., cisplatin, mechlorethamine, cyclophosphamide, ifosfamide, melphalan, chorambucil, busulfan, thiotepa, carmustine, lomustine, carboplatin, dacarbazine, procarbazine, temozolomidel (TEMODAR)), DNA strand break inducing agents (e.g., bleomycin, doxorubicin, daunorubicin, idarubicin, mitomycin C), and radiation therapy.

The anti-cancer agent may be docetaxel (TAXOTERE) (e.g., in non-small cell lung carcinoma), cisplatin (e.g., in lymphoma and melanoma), gemcitabine, pemetrexed (ALIMTA), erlotinib (TARCEVA), gefitinib (IRESSA), temozolomide (TEMODAR), carboplatin, cyclophosphamide, 5-fluorouracil, dacarbazine, paclitaxel, thalidomide, or doxorubicin.

Suitable anti-cancer compounds that may be used in the invention include Acivicin; Aclarubicin; Acodazole Hydrochloride; Acronine; Adozelesin; Aldesleukin; Altretamine; Ambomycin; Ametantrone Acetate; Aminoglutethimide; Amsacrine; Anastrozole; Anthramycin; Asparaginase; Asperlin; Azacitidine; Azetepa; Azotomycin; Batimastat; Benzodepa; Bicalutamide; Bisantrene Hydrochloride; Bisnafide Dimesylate; Bizelesin; Bleomycin Sulfate; Brequinar Sodium; Bropirimine; Busulfan; Cactinomycin; Calusterone; Caracemide; Carbetimer; Carboplatin; Carmustine; Carubicin Hydrochloride; Carzelesin; Cedefingol; Chlorambucil; Cirolemycin; Cisplatin; Cladribine; Crisnatol Mesylate; Cyclophosphamide; Cytarabine; Dacarbazine; Dactinomycin; Daunorubicin Hydrochloride; Decitabine; Dexormaplatin; Dezaguanine; Dezaguanine Mesylate; Diaziquone; Docetaxel; Doxorubicin; Doxorubicin Hydrochloride; Droloxifene; Droloxifene Citrate; Dromostanolone Propionate; Duazomycin; Edatrexate; Eflornithine Hydrochloride; Elsamitrucin; Enloplatin; Enpromate; Epipropidine; Epirubicin Hydrochloride; Erbulozole; Esorubicin Hydrochloride; Estramustine; Estramustine Phosphate Sodium; Etanidazole; Etoposide; Etoposide Phosphate; Etoprine; Fadrozole Hydrochloride; Fazarabine; Fenretinide; Floxuridine; Fludarabine Phosphate; Fluorouracil; Flurocitabine; Fosquidone; Fostriecin Sodium; Gemcitabine; Gemcitabine Hydrochloride; Hydroxyurea; Idarubicin Hydrochloride; Ifosfamide; Umofosine; Interferon Alfa-2a; Interferon Alfa-2b; Interferon Alfa-nl; Interferon Alfa-n3; Interferon Beta-I a; Interferon Gamma-I b; Iproplatin; Irinotecan Hydrochloride; Lanreotide Acetate; Letrozole; Leuprolide Acetate; Liarozole Hydrochloride; Lometrexol Sodium; Lomustine; Losoxantrone Hydrochloride; Masoprocol; Maytansine; Mechlorethamine Hydrochloride; Megestrol Acetate; Melengestrol Acetate; Melphalan; Menogaril; Mercaptopurine;

Methotrexate; Methotrexate Sodium; Metoprine; Meturedepa; Mitindomide; Mitocarcin; Mitocromin; Mitogillin; Mitomalcin; Mitomycin; Mitosper; Mitotane; Mitoxantrone Hydrochloride; Mycophenolic Acid; Nocodazole; Nogalamycin; Ormaplatin; Oxisuran; Paclitaxel; Pegaspargase; Peliomycin; Pentamustine; Peplomycin Sulfate; Perfosfamide; Pipobroman; Piposulfan; Piroxantrone Hydrochloride; Plicamycin; Plomestane; Porfimer Sodium; Porfiromycin; Prednimustine; Procarbazine Hydrochloride; Puromycin; Puromycin Hydrochloride; Pyrazofurin; Riboprine; Rogletimide; Safingol; Safingol Hydrochloride; Semustine; Simtrazene; Sparfosate Sodium; Sparsomycin; Spirogermanium Hydrochloride; Spiromustine; Spiroplatin; Streptonigrin; Streptozocin; Sulofenur; Talisomycin; Taxol; Taxotere; Tecogalan Sodium; Tegafur; Teloxantrone Hydrochloride; Temoporfin; Teniposide; Teroxirone; Testolactone; Thiamiprine; Thioguanine; Thiotepa; Tiazofurin; Tirapazamine; Topotecan Hydrochloride; Toremifene Citrate; Trestolone Acetate; Triciribine Phosphate; Trimetrexate; Trimetrexate Glucuronate; Triptorelin; Tubulozole Hydrochloride; Uracil Mustard; Uredepa; Vapreotide; Verteporfin; Vinblastine Sulfate; Vincristine Sulfate; Vindesine; Vindesine Sulfate; Vinepidine Sulfate; Vinglycinate Sulfate; Vinleurosine Sulfate; Vinorelbine Tartrate; Vinrosidine Sulfate; Vinzolidine Sulfate; Vorozole; Zeniplatin; Zinostatin; Zorubicin Hydrochloride.

Other anti-cancer drugs include: 20-epi-l,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-poφ hyrin;

cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; cohagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; decitabine; dehydrodidemnin B; deslorelin; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, *A-;* irinotecan; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide + estrogen + progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A + myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anti cancer compound; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone + pentazocine; napavin; naphterpin; nartograstim; nedaplatin;

nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone Bl; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine, senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thalidomide; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene dichloride; topotecan; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene

therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; zinostatin stimalamer.

The subjects can also be administered the agent in combination with an anti-cancer supplementary potentiating compound. Examples of anti-cancer supplementary potentiating compounds include: Tricyclic anti-depressant drugs (e.g., imipramine, desipramine, amitryptyline, clomipramine, trimipramine, doxepin, nortriptyline, protriptyline, amoxapine and maprotiline); non-tricyclic anti-depressant drugs (e.g., sertraline, trazodone and citalopram); Ca^{++} antagonists (e.g., verapamil, nifedipine, nitrendipine and caroverine); Calmodulin inhibitors (e.g., prenylamine, trifluoroperazine and clomipramine); Amphotericin B; Triparanol analogues (e.g., tamoxifen); antiarrhythmic drugs (e.g., quinidine); antihypertensive drugs (e.g., reserpine); Thiol depleters (e.g., buthionine and sulfoximine) and multiple drug resistance reducing compounds such as Cremaphor EL.

Other compounds which are useful in combination therapy for the purpose of the invention include the antiproliferation compound, Piritrexim Isethionate; the antiprostatic hypertrophy compound, Sitogluside; the benign prostatic hyperplasia therapy compound, Tamsulosin Hydrochloride; the prostate growth inhibitor, Pentomone; radioactive compounds such as Fibrinogen I 125, Fludeoxyglucose F 18, Fluorodopa F 18, Insulin I 125, Insulin I 131, Iobenguane I 123, Iodipamide Sodium I 131, Iodoantipyrine I 131, Iodocholesterol I 131, Iodohippurate Sodium I 123, Iodohippurate Sodium I 125, Iodohippurate Sodium I 131, Iodopyracet I 125, Iodopyracet I 131, Iofetamine Hydrochloride I 123, Iomethin I 125, Iomethin I 131, Iothalamate Sodium I 125, Iothalamate Sodium I 131, Iotyrosine I 131, Liothyronine I 125, Liothyronine I 131, Merisoprol Acetate Hg 197, Merisoprol Acetate Hg 203, Merisoprol Hg 197, Selenomethionine Se 75, Technetium Tc 99m Antimony Trisulfide Colloid, Technetium Tc 99m Bicisate, Technetium Tc 99m Disofenin, Technetium Tc 99m Etidronate, Technetium Tc 99m Exametazime, Technetium Tc 99m Furifosmin, Technetium Tc 99m Gluceptate, Technetium Tc 99m Lidofenin, Technetium Tc 99m Mebrofenin, Technetium Tc 99m Medronate, Technetium Tc 99m Medronate Disodium, Technetium Tc 99m Mertiatide, Technetium Tc 99m Oxidronate, Technetium Tc 99m Pentetate, Technetium Tc 99m Pentetate Calcium Trisodium, Technetium Tc 99m Sestamibi, Technetium Tc 99m Siboroxime, Technetium Tc 99m Succimer, Technetium Tc 99m Sulfur Colloid, Technetium Tc 99m Teboroxime, Technetium Tc 99m Terrofosmin, Technetium Tc 99m Tiatide, Thyroxine I 125, Thyroxine I 131, Tolpovidone I 131, Triolein I 125 and Triolein I 131.

In important embodiments, the agents of the invention are administered together with one or more anti-cancer compounds selected from the group consisting of aldesleukin, asparaginase, bleomycin sulfate, carboplatin, chlorambucil, cisplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin hydrochloride, docetaxel, doxorubicin, doxorubicin hydrochloride, epirubicin hydrochloride, etoposide, etoposide phosphate, floxuridine, fludarabine, fluorouracil, gemcitabine, gemcitabine hydrochloride, hydroxyurea, idarubicin hydrochloride, ifosfamide, interferons, interferon-α2a, interferon-α2b, interferon-αn3, interferon-αlb, interleukins, irinotecan, mechlorethamine hydrochloride, melphalan, mercatopurine, methotrexate, methotrexate sodium, mitomycin, mitoxantrone, paclitaxel, pegaspargase, pentostatin, prednisone, profimer sodium, procabazine hydrochloride, taxol, taxotere, teniposide, topotecan hydrochloride, vinblastine sulfate, vincristine sulfate vinorelbine tartrate, annonaceous acetogenins; asimicin; rolliniastatin; guanacone, squamocin, bullatacin; squamotacin; taxanes; paclitaxel; gemcitabine; methotrexate FR-900482; FK-973; FR-66979; FK-317; 5-FU; FUDR; FdUMP; Hydroxyurea; Docetaxel; discodermolide; epothilones; vincristine; vinblastine; vinorelbine; meta-pac; irinotecan; SN-38; 10-OH campto; topotecan; etoposide; adriamycin; flavopiridol; Cis-Pt; carbo-Pt; bleomycin; mitomycin C; mithramycin; capecitabine; cytarabine; 2-C1-2'deoxyadenosine; Fludarabine-P θ 4; mitoxantrone; mitozolomide; Pentostatin; Tomudex.

One particularly preferred class of anti-cancer agents are taxanes (e.g., paclitaxel and docetaxel). Another important category of anticancer agent is annonaceous acetogenin.

Other cancer therapies include hormonal manipulation, particularly for breast and gynecological cancers. Agents of the invention are also useful in combination with tamoxifen or aromatase inhibitor arimidex (i.e., anastrozole), or simply for disorders responsive to either (e.g., breast cancer).

The agents of the invention can also be combined, and/or administered substantially simultaneously, with enzyme inhibitor agents such as CDK inhibitors, tyrosine kinase inhibitors, MAP kinase inhibitors, and EGFR inhibitors (e.g., C225). The tyrosine kinase inhibitor may be Gleevac (imatinib mesylate), Genistein (4',5,7-trihydroxyisoflavone), Tyrphostin 25 (3,4,5-trihydroxyphenyl), methylene]-propanedinitrile, Herbimycin A, Daidzein (4',7-dihydroxyisoflavone), AG-126, trans-l-(3'-carboxy-4'-hydroxyphenyl)-2-(2",5"-dihydroxy-phenyl)ethane, or HDBA (2-Hydroxy5-(2,5-Dihydroxybenzylamino)-2-hydroxybenzoic acid. The CDK inhibitor may be p21, p27, p57, pi5, pi6, pi8, or pl9. The MAP kinase inhibitor may be KY12420 ($C_{23}H_{24}O_8$), CNI-1493, PD98059, or 4-(4-

Fluorophenyl)-2-(4-methylsulfinyl phenyl)-5-(4-pyridyl) lH-imidazoIe. The EGFR inhibitor may be erlotinib (TARCEVA), gefitinib (IRESSA), WHI-P97 (quinazoline derivative), LFM-A12 (leflunomide metabolite analog), ABX-EGF, lapatinib, canertinib, ZD-6474 (ZACTIMA), AEE788, and AGl 458.

The agents of the invention can also be used in combination with VEGF inhibitors or antagonists. VEGF inhibitors include bevacizumab (AVASTIN), ranibizumab (LUCENTIS), pegaptanib (MACUGEN), sorafenib, sunitinib (SUTENT), vatalanib, ZD-6474 (ZACTIMA), anecortave (RETAANE), squalamine lactate, and semaphorin.

The goal of cancer immunotherapy is to augment a subject's immune response to an established tumor. Different types of cells that can kill tumor targets *in vitro* and *in vivo* have been identified: natural killer cells (NK cells), cytolytic T lymphocytes (CTLs), lymphokine-activated killer cells (LAKs), activated macrophages, and neutrophils. NK cells can kill tumor cells without having been previously sensitized to specific antigens, and the activity does not require the presence of class I antigens encoded by the major histocompatibility complex (MHC) on target cells. NK cells are thought to participate in the control of nascent tumors and in the control of metastatic growth. In contrast to NK cells, CTLs can kill tumor cells only after they have been sensitized to tumor antigens and when the target antigen is expressed on the tumor cells that also express MHC class I. CTLs are thought to be effector cells in the rejection of transplanted tumors and of tumors caused by DNA viruses. LAK cells are a subset of null lymphocytes distinct from the NK and CTL populations. Activated macrophages and neutrophils can directly kill tumor cells in a manner that is not antigen dependent nor MHC restricted. In addition, neutrophils can inhibit tumor growth by killing endothelial cells of the vasculature that provide blood supply to the tumor. Thus, activated macrophages and neutrophils are thought to decrease the growth rate of the tumors they infiltrate.

Agents of the invention when administered to tumor-bearing mice, rapidly stimulate the production of growth factors, cytokines and chemokines. These mediators collectively stimulate the proliferation, activation and chemoattraction to the tumor microenvironment of effector cells involved in both non-adaptive (innate) and immune lysis or growth inhibition of tumor cells. The immune and non-immune effector cell populations mobilized and/or activated by agents of the invention enhance the tumor suppressive effects of anti-cancer antibodies.

Examples of effector cells involved in the anti-tumor effects of the agents are given below. Although not intending to be bound by any particular mechanism, a brief description of how each cell type can cooperate with tumor-specific antibodies in the lysis or growth inhibition of tumor cells is provided herein.

Tumor-infiltrating T cells, including cytotoxic T lymphocytes (CTL), that either lyse or inhibit tumor growth will suppress tumors by a mechanism of antigen-recognition that is different from that of antibodies. Thus, tumor-specific T cells can augment tumor cell lysis or growth inhibition initiated by antibody-based therapeutics.

Macrophage/monocyte, neutrophil, eosinophil, natural killer cells, and lymphokine activated killer cells are also activated by the agents. Individually or collectively, these effector cell types can either lyse tumor cells or suppress their growth in ligand-receptor mediated interactions that lack immunological specificity. The activities of these cells can account for the innate or non-adaptive immune responses against tumors stimulated by the agents. In addition, all of these cell types possess receptors that bind to the Fc portion of immunoglobulin and are referred to as Fc receptors. Fc receptors can bind to antibodies that are specifically bound to tumor cells by their antigen-binding regions. Therefore, since each effector cell possesses cytotoxicity or growth inhibitory activity against tumor cells, the antibody-mediated interaction targets this activity specifically against the tumor. The mechanism can therefore increase the efficiency with which these otherwise non-specific effector cells suppress tumor growth. The process is frequently referred to as antibody dependent cell-mediated cytotoxicity (ADCC).

Thus, the invention provides methods for the treatment of cancer in a subject using agents of the invention in combination with cancer-specific antibodies. In one embodiment, the combination is synergistic, resulting in a greater effect than the additive effect of the agents when used separately. In other embodiments, the combination is additive.

Antibodies specific for tumor or cancer antigens can suppress tumor growth *in vivo* via a variety of mechanisms. Antibody dependent cell-mediated cytotoxicity, complement mediated cell lysis, targeting of chemically linked toxins, inhibition of tumor cell division, and induction of tumor cell apoptosis have all been described as mechanisms by which antibodies specific for tumor or cancer antigens suppress tumor growth in the treatment of cancer. Although antibody-based treatments (immunotherapy) for cancer can be effective, they do not completely suppress tumor development and progression in all subjects.

The invention further provides a method for stimulating ADCC in a subject. The method comprises administering an anti-cancer antibody or antibody fragment and an agent of the invention to a subject in need thereof (e.g., a subject having a cancer) in an amount effective to stimulate antibody dependent cell-mediated cytotoxicity in the subject. In some embodiments, the amount effective to stimulate antibody dependent cell-mediated cytotoxicity is a synergistic amount.

The invention also contemplates the use of the agents of the invention together with antigens such as cancer antigens and microbial agents.

Microbial antigens that can be used in the methods of the invention include whole bacteria, whole virus, whole fungi, whole parasites, and fragments thereof. Examples include non-infectious human papillomavirus-like particles (VLP) (which can be used as a cancer antigen as well, particularly for cervical cancer); and the like.

The invention can be used to treat cancers that are immunogenic. Cancers that are immunogenic are cancers that are known to (or likely to) express immunogens on their surface or upon cell death. These immunogens are in vivo endogenous sources of cancer antigens and their release can be exploited by the methods of the invention in order to treat the cancer. Examples of immunogenic cancers include those listed in Table 2, including malignant melanoma and renal cell cancer.

The terms "cancer antigen" and "tumor antigen" are used interchangeably. A cancer antigen as used herein is a compound differentially associated with a tumor or cancer, preferably at the cell surface of a tumor or cancer cell, that is capable of invoking an immune response. The cancer antigen may be peptide in nature but it is not so limited. As an example, the antigen may be a lipid antigen, as described in U.S. Patents US 5,679,347, issued on October 21, 1997 and US 6,238,676 Bl, issued on May 29, 2001. If the antigen is a peptide, then it invokes an immune response when it is presented (in a digested form) on the surface of an antigen presenting cell in the context of an MHC molecule. If the antigen is a lipid, then it invokes an immune response when it is presented in the context of a CDl molecule. Cancer antigens can be prepared from cancer cells either by preparing crude extracts of cancer cells, for example, as described in Cohen, et al., 1994, *Cancer Research,* 54: 1055, by partially purifying the antigens, by recombinant technology, or by de novo synthesis of known antigens. Cancer antigens include but are not limited to antigens that are recombinantly expressed, an immunogenic portion of, or a whole tumor or cancer. Such antigens can be isolated or prepared recombinantly or by any other means known in the art.

A cancer antigen encompasses antigens that are differentially expressed between cancer and normal cells. Due to this differential expression, these antigens can be targeted in anti-tumor therapies. Cancer antigens may be expressed in a regulated manner in normal cells. For example, they may be expressed only at certain stages of differentiation or at certain points in development of the organism or cell. Some are temporally expressed as embryonic and fetal antigens. Still others are never expressed in normal cells, or their expression in such cells is so low as to be undetectable.

Other cancer antigens are encoded by mutant cellular genes, such as oncogenes (e.g., activated ras oncogene), suppressor genes (e.g., mutant ρ53), fusion proteins resulting from internal deletions or chromosomal translocations. Still other cancer antigens can be encoded by viral genes such as those carried on RNA and DNA tumor viruses.

Examples of cancer antigens include HER 2 (pi 85), CD20, CD33, GD3 ganglioside, GD2 ganglioside, carcinoembryonic antigen (CEA), CD22, milk mucin core protein, TAG-72, Lewis A antigen, ovarian associated antigens such as OV-TL3 and MOvI 8, high Mr melanoma antigens recognized by antibody 9.2.27, HMFG-2, SM-3, B72.3, PR5C5, PR4D2, and the like. Other cancer antigens are described in U.S. Pat. No. 5,776,427. Still other cancer antigens are listed in Table 2.

Further examples include MAGE, MART-1/Melan-A, gplOO, Dipeptidyl peptidase IV (DPP-FV), adenosine deaminase-binding protein (ADAbp), FAP, cyclophilin b, Colorectal associated antigen (CRC)-COl 7-1A/GA733, Carcinoembryonic Antigen (CEA) and its immunogenic epitopes CAP-I and CAP-2, etv6, amll, Prostate Specific Antigen (PSA) and its immunogenic epitopes PSA-I, PSA-2, and PSA-3, prostate-specific membrane antigen (PSMA), T-cell receptor/CD3-zeta chain, MAGE-family of tumor antigens (e.g., MAGE-Al, MAGE-A2, MAGE- A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-Al 0, MAGE-Al 1, MAGE-Al 2, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE-Cl, MAGE-C2, MAGE-C3, MAGE-C4, MAGE-C5), GAGE-family of tumor antigens (e.g., GAGE-I, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, GAGE-9), BAGE, RAGE, LAGE-I, NAG, GnT-V, MUM-I, CDK4, tyrosinase, p53, MUC family, HER2/neu, p21ras, RCAS 1, α-fetoprotein, E-cadherin, α-catenin, β-catenin and γ-catenin, p 120ctn, gp 100^{Pmel17}, PRAME, NY-ESO- 1, cdc27, adenomatous polyposis coli protein (APC), fodrin, Connexin 37, Ig-idiotype, pi 5, gp75, GM2 and GD2 gangliosides, viral products such as human papilloma virus proteins, Smad family of tumor antigens, lmp-1, PlA, EBV-encoded nuclear antigen (EBNA)-I, brain

glycogen phosphorylase, SSX-I, SSX-2 (HOM-MEL-40), SSX-I, SSX-4, SSX-5, SCP-I and CT-7, CD20 and c-erbB-2.

These antigens can be classified as indicated in Table 2.

Table 2. Classification of cancer antigens

Table 2a. Proteins encoded by genes that have undergone chromosomal alteration in lymphoma and leukemia

Genes	Disease
Activation of quiescent genes	
BCL-1 and IgH	Mantel cell lymphoma
BCL-2 and IgH	Follicular lymphoma
BCL-6	Diffuse large B-cell lymphoma
TAL-1 and TCRδ or SIL	T-cell acute lymphoblastic leukemia
c-MYC and IgH or IgL	Burkitt lymphoma
MUN/IRF4 and IgH	Myeloma
PAX-5 (BSAP)	Immunocytoma
Creation of fusion genes	
RARα, PML, PLZF, NPM or NuMA	Acute promyelocytic leukemia
BCR and ABL	Chronic myeloid/acute lymphoblastic leukemia
MLL (HRX)	Acute leukemia
E2A and PBX or HLF	B-cell acute lymphoblastic leukemia
NPM, ALK	Anaplastic large cell leukemia
NPM, MLF-1	Myelodysplastic syndrome/acute myeloid leukemia

Adapted from Falini B. and Mason, D.Y. (2002) Blood 99: 409-426

Table 2b. Proteins specific to a tissue or cell lineage

Protein	Disease
Cell-surface proteins	
CD20, CD22	Non-Hodgkin's lymphoma, B-cell lymphoma, Chronic lymphocytic leukemia (CLL)
CD52	B-cell CLL
CD33	Acute myelogenous leukemia (AML)
CD10 (gp100)	Common (pre-B) acute lymphocytic leukemia and malignant melanoma
CD3/T-cell receptor (TCR)	T-cell lymphoma and leukemia
CD79/B-cell receptor (BCR)	B-cell lymphoma and leukemia
CD26	Epithelial and lymphoid malignancies
Human leukocyte antigen (HLA)-DR, HLA-DP, and HLA-DQ	Lymphoid malignancies
RCAS1	Gynecological carcinomas, bilary adenocarcinomas and ductal adenocarcinomas of the pancreas

Prostate specific membrane antigen	Prostate cancer
Epidermal growth factor receptors (high expression	
EGFR (HER1 or erbB1) and EGFRvIII	Brain, lung, breast, prostate and stomach cancer
erbB2 (HER2 or HER2/neu)	Breast cancer and gastric cancer
erbB3 (HER3)	Adenocarcinoma
erbB4 (HER4)	Breast cancer
Cell-associated proteins	
Tyrosinase, Melan-A/MART-1, tyrosinase related protein (TRP)-1/gp75	Malignant melanoma
Polymorphic epithelial mucin (PEM)	Breast tumors
Human epithelial mucin (MUC1)	Breast, ovarian, colon and lung cancers
Secreted proteins	
Monoclonal immunoglobulin	Multiple myeloma and plasmacytoma
Immunoglobulin light chains	Multiple Myeloma
α-fetoprotein	Liver carcinoma
Kallikreins 6 and 10	Ovarian cancer
Gastrin-releasing peptide/bombesin	Lung carcinoma
Prostate specific antigen	Prostate cancer

Table 2c. Cancer testis (CT) antigens*

These antigens include MAGE-A1, -A3, -A6, -A12, BAGE, GAGE, HAGE, LAGE-1, NY-ESO-1, RAGE, SSX-1, -2, -3, -4, -5, -6, -7, -8, -9, HOM-TES-14/SCP-1, HOM-TES-85 and PRAME.

* These antigens are expressed in some normal tissues such as testis and in some cases placenta. Their expression is common in tumors of diverse lineages and as a group the antigens form targets for immunotherapy. Examples of tumor expression of CT antigens are as follows.

Protein	**Disease**
SSX-2, and -4	Neuroblastoma
SSX-2 (HOM-MEL-40), MAGE, GAGE, BAGE and PRAME	Malignant melanoma
HOM-TES-14/SCP-1	Meningioma
SSX-4	Oligodendrioglioma
HOM-TES-14/SCP-1, MAGE-3 and SSX-4	Astrocytoma
SSX member	Head and neck cancer, ovarian cancer, lymphoid tumors, colorectal cancer and breast cancer
RAGE-1, -2, -4, GAGE-1, -2, -3, -4, -5, -6, -7 and -8	Head and neck squamous cell carcinoma (HNSCC)
HOM-TES14/SCP-1, SSX-1, PRAME and CT-7	Non-Hodgkin's lymphoma
PRAME	Acute lymphoblastic leukemia (ALL), acute

	myelogenous leukemia (AML) and chronic lymphocytic leukemia (CLL)

Table 2d. Proteins not-specific to a tissue or cell lineage*

Carcinoembryonic antigen (CEA) family: CD66a, CD66b, CD66c, CD66d and CD66e. *These antigens can be expressed in many different malignant tumors and can be targeted by immunotherapy.

Table 2e. Viral proteins

Human papilloma virus protein (cervical cancer) EBV-encoded nuclear antigen (EBNA)-I (lymphomas of neck and oral cancer)

Table 2f. Mutated or aberrantly expressed molecules

CDK4 and beta-catenin in melanoma

Cancer or tumor antigens can also be classified according to the cancer or tumor they are associated with (i.e., expressed by). Cancers or tumors associated with tumor antigens include acute lymphoblastic leukemia (etv6; amll ; cyclophilin b), B cell lymphoma (Ig-idiotype); Burkitt's (Non-Hodgkin's) lymphoma (CD20); glioma (E-cadherin; α-catenin; β-catenin; γ-catenin; pl20ctn), bladder cancer (p21ras), biliary cancer (p21ras), breast cancer (MUC family; HER2/neu; c-erbB-2), cervical carcinoma (p53; p21ras), colon carcinoma (p21ras; HER2/neu; c-erbB-2; MUC family), colorectal cancer (Colorectal associated antigen (CRC)--CO17-1A/GA733; APC), choriocarcinoma (CEA), epithelial cell-cancer (cyclophilin b), gastric cancer (HER2/neu; c-erbB-2; ga733 glycoprotein), hepatocellular cancer (α-fetoprotein), Hodgkin's lymphoma (lmp-1; EBNA-I), lung cancer (CEA; MAGE-3; NY-ESO-I), lymphoid cell-derived leukemia (cyclophilin b), melanoma (pi 5 protein, gp75, oncofetal antigen, GM2 and GD2 gangliosides), myeloma (MUC family; p21ras), non-small cell lung carcinoma (HER2/neu; c-erbB-2), nasopharyngeal cancer (lmp-1; EBNA-I), ovarian cancer (MUC family; HER2/neu; c-erbB-2), prostate cancer (Prostate Specific Antigen (PSA) and its immunogenic epitopes PSA-I, PSA-2, and PSA-3; PSMA; HER2/neu; c-erbB-2), pancreatic cancer (p2 Iras; MUC family; HER2/neu; c-erbB-2; ga733 glycoprotein), renal (HER2/neu; c-erbB-2), squamous cell cancers of cervix and esophagus (viral products such as human papilloma virus proteins and non-infectious particles), testicular cancer (NY-ESO-I), T cell leukemia (HTLV-I epitopes), and melanoma (Melan-A/MART-1; cdc27; MAGE-3; p21ras; gplOO^{Pmel117}).

For examples of tumor antigens which bind to either or both MHC class I and MHC class II molecules, see the following references: Coulie, *Stem Cells* 13:393-403, 1995; Traversari et al., *J. Exp. Med.* 176:1453-1457, 1992; Chaux et al., *J. Immunol.* 163:2928-2936, 1999; Fujie et al., *Int. J. Cancer* 80:169-172, 1999; Tanzarella et al., *Cancer Res.* 59:2668-2674, 1999; van der Bruggen et al., *Eur. J. Immunol.* 24:2134-2140, 1994; Chaux et al., *J. Exp. Med.* 189:767-778, 1999; Kawashima et *a\, Hum. Immunol.* 59:1-14, 1998; Tahara et al., *Clin. Cancer Res.* 5:2236-2241, 1999; Gaugler et al., *J. Exp. Med.* 179:921-930, 1994; van der Bruggen et al., *Eur. J. Immunol.* 24:3038-3043, 1994; Tanaka et al., *Cancer Res.* 57:4465-4468, 1997; Oiso et al., *Int. J. Cancer* 81:387-394, 1999; Herman et al., *Immunogenetics* 43:377-383, 1996; Manici et al., *J. Exp. Med.* 189:871-876, 1999; Duffour et al., *Eur. J. Immunol.* 29:3329-3337, 1999; Zorn et al., *Eur. J. Immunol.* 29:602-607, 1999; Huang et al., *J. Immunol.* 162:6849-6854, 1999; Boel et al., *Immunity* 2:167-175, 1995; Van den Eynde et al., *J. Exp. Med.* 182:689-698, 1995; De Backer et al., *Cancer Res.* 59:3157-3165, 1999; Jager et al., *J. Exp. Med.* 187:265-270, 1998; Wang et al., *J. Immunol.* 161:3596-3606, 1998; Aarnoudse et al., *Int. J. Cancer* 82:442-448, 1999; Guilloux et al., *J. Exp. Med.* 183:1173-1 183, 1996; Lupetti et *si., J. Exp. Med.* 188:1005-1016, 1998; Wδlfel et al., £Mr. *J. Immunol.* 24:759-764, 1994; Skipper et al., *J. Exp. Med.* 183:527-534, 1996; Kang et al., *J. Immunol.* 155:1343-1348, 1995; Morel et al., *Int. J. Cancer* 83:755-759, 1999; Brichard et al., *Eur. J. Immunol.* 26:224-230, 1996; Kittlesen et al., *J. Immunol.* 160:2099-2106, 1998; Kawakami et al., *J. Immunol.* 161:6985-6992, 1998; Topalian et al., *J. Exp. Med.* 183:1965-1971, 1996; Kobayashi et al., *Cancer Research* 58:296-301, 1998; Kawakami et al., *J. Immunol.* 154:3961-3968, 1995; Tsai et al., *J. Immunol.* 158:1796-1802, 1997; Cox et al., *Science* 264:716-719, 1994; Kawakami et al., *Proc. Natl. Acad. Sci. USA* 91:6458-6462, 1994; Skipper et al., *J. Immunol.* 157:5027-5033, 1996; Robbins et al., *J. Immunol.* 159:303-308, 1997; Castelli et al, *J. Immunol.* 162:1739-1748, 1999; Kawakami et al., *J. Exp. Med.* 180:347-352, 1994; Castelli et al., *J. Exp. Med.* 181:363-368, 1995; Schneider et al., *Int. J. Cancer* 75:451-458, 1998; Wang et al., *J. Exp. Med.* 183:1 131-1 140, 1996; Wang et al., *J. Exp. Med.* 184:2207-2216, 1996; Parkhurst et al., *Cancer Research* 58:4895-4901, 1998; Tsang et al., *J. Natl Cancer Inst* 87:982-990, 1995; Correale et al., *J Natl Cancer Inst* 89:293-300, 1997; Coulie et al., *Proc. Natl. Acad. Sci. USA* 92:7976-7980, 1995; Wδlfel et al., *Science* 269:1281-1284, 1995; Robbins et al., *J. Exp. Med.* 183:1185-1192, 1996; Brandle et al., *J. Exp. Med.* 183:2501-2508, 1996; ten Bosch et al., *Blood* 88:3522-3527, 1996; Mandruzzato et al., *J. Exp. Med.* 186:785-793, 1997; Gueguen et al., *J. Immunol.* 160:6188-

6194, 1998; Gjertsen et al., *Int. J. Cancer* 72:784-790, 1997; Gaudin et al., *J. Immunol.* 162:1730-1738, 1999; Chiari et al., *Cancer Res.* 59:5785-5792, 1999; Hogan et al., *Cancer Res.* 58:5144-5150, 1998; Pieper et al., *J. Exp. Med.* 189:757-765, 1999; Wang et al., *Science* 284:1351-1354, 1999; Fisk et al., *J. Exp. Med.* 181:2109-21 17, 1995; Brossart et al., *Cancer Res.* 58:732-736, 1998; Rδpke et al., *Proc. Natl. Acad. ScL USA* 93:14704-14707, 1996; Dceda et al., *Immunity* 6:199-208, 1997; Ronsin et al., *J. Immunol.* 163:483-490, 1999; Vonderheide et al., *Immunity* 10:673-679,1999. These antigens as well as others are disclosed in PCT Application PCT/US98/18601.

In some preferred embodiments, the cancer antigen is VEGF, Anti-idiotypic mAb (GD3 ganglioside mimic), CD20, CD52, Anti-idiotypic mAb (CEA mimic), ERBB2, EGFR, CD22, ERBB2 X CD65 (fcγRI), EpCam, PEM and CD33.

The invention therefore provides methods for using an agent of the invention in combination with various vaccines either currently being used or in development, whether intended for human or non-human subjects. Examples of vaccines for human subjects and directed to infectious diseases include the combined diphtheria and tetanus toxoids vaccine; pertussis whole cell vaccine; the inactivated influenza vaccine; the 23-valent pneumococcal vaccine; the live measles vaccine; the live mumps vaccine; live rubella vaccine; Bacille Calmette-Guerin (BCG) tuberculosis vaccine; hepatitis A vaccine; hepatitis B vaccine; hepatitis C vaccine; rabies vaccine (e.g., human diploid cell vaccine); inactivated polio vaccine; meningococcal polysaccharide vaccine; quadrivalent meningococcal vaccine; yellow fever live virus vaccine; typhoid killed whole cell vaccine; cholera vaccine; Japanese B encephalitis killed virus vaccine; adenovirus vaccine; cytomegalovirus vaccine; rotavirus vaccine; varicella vaccine; anthrax vaccine; and small pox vaccine.

The vaccine methods and compositions described herein similarly envision the use of nucleic acid based vaccines in addition to peptide based vaccines. The art is familiar with nucleic acid based vaccines.

In still another aspect, the invention provides a method for shortening a vaccination course. As used herein, "shortening a vaccination course" refers to reducing either the number of vaccine administrations (e.g., by injection) or the time between vaccine administrations. This is accomplished by stimulating a more robust immune response in the subject. The method may involve, in one embodiment, administering to a subject in need of immunization an agent of the invention in an amount effective to induce an antigen-specific immune response to a vaccine administered in a vaccination course, wherein the vaccination

course is shortened by at least one immunization. In other embodiments, the vaccination course is shortened by one immunization, two immunizations, three immunizations, or more. The method may involve, in another embodiment, administering to a subject in need of immunization an agent of the invention in an amount effective to induce an antigen-specific immune response to a vaccine administered in a vaccination course, wherein the vaccination course is shortened by at least one day. In other embodiments, the vaccination course is shortened by one day, two days, three days, four days, five days, six days, one week, two weeks, three weeks, four weeks, one month, two months or more. In one embodiment, an agent of the invention is administered substantially simultaneously with the vaccine.

Immunizations that can be modified in this way include but are not limited to newborn immunizations for HBV; immunizations at for example two months of age for Polio, DTaP, Hib, HBV, Pneumococcus; immunizations at for example four months of age for Polio, DTaP, Hib, Pneumococcus; immunizations at for example six months of age for Polio, DTaP, Hib, HBV, Pneumococcus; immunizations at for example 12-15 months of age for Hib, Pneumococcus, MMR, Varicella; immunizations at for example 15-18 months of age for DtaP; immunizations at for example 4-6 years of age for Polio, DPT, MMR; immunizations at for example 11-12 years of age for MMR; immunizations at for example 14-16 years of age for tetanus-diphtheria (i.e., Td) (with a repeat as a booster every 10 years).

As an example, a recommended vaccination course for tetanus/diphtheria includes a primary immunization series given in adults if not received as a child, followed by routine booster doses of tetanus-diphtheria (Td) every 10 years. The method of the invention will allow for a shortened series of vaccinations at the first time point, and may in some instances obviate the need for subsequent booster shoots. As another example, hepatitis vaccination commonly requires three administrations spaced at least two weeks, and sometimes one month, apart in order to develop full immunity. Using the methods of the invention, it is possible either to reduce the number of injections from three to two or one, or to reduce the time in between injections from weeks or months to days or weeks.

Vaccination courses that can be shortened by the method of the invention include but are not limited to: HBV: Hepatitis B vaccine (3 total doses currently recommended); Polio: Inactivated polio vaccine (4 total doses currently recommended); DTaP: Diphtheria/tetanus/acellular Pertussis (3-in-l vaccine; 5 total doses currently recommended); Hib: Haemophilus influenzae type b conjugate vaccine (4 total doses currently recommended); Pneumococcus (Prevnar): Protects against certain forms of Strep.

Pneumoniae (3 total doses recommended); MMR: measles/mumps/rubella (3-in-l vaccine; 2 total doses recommended); Td: Adult tetanus/diphtheria (2-in-l vaccine; for use in people over age 7). In another embodiment, an agent of the invention can be used together with oral polio vaccine.

Certain methods and compositions comprise, in addition to the agents of the invention, an antibody or fragment thereof. The invention embraces the use of antibodies of all isotypes including IgM, IgAl, IgA2, slgA, IgD, IgE, IgGl, IgG2, IgG3, and IgG4, having light chains that are either kappa or lambda chains.

The antibodies that can be used with the agents of the invention include those useful in cancer and infectious disease as well as other disorders for which antibodies and antigens have been identified and which would benefit from an enhanced immune response.

The antibodies or fragments thereof useful in the invention can be specific for any component of a particular target. Accordingly, the antibody can recognize and bind to proteins, lipids, carbohydrates, DNA, RNA, and any combination of these in molecular or supra-molecular structures (e.g., cell organelles such as mitochondria or ribosomes). The antibody or fragment thereof can also recognize a modification of the tumor cell, such as e.g., chemical modifications, or genetic modifications made by transfection *ex vivo* or *in vivo* with DNA or RNA. As used herein, the terms "antibody" and "immunoglobulin" are used interchangeably.

Bispecific antibodies can also be used in the invention. A bispecific antibody is one having one variable region that specifically recognizes a tumor antigen and the other variable region that specifically recognizes an antigenic epitope of a host immune effector cell that has lytic or growth inhibitory activity against the tumor. Bispecific and multispecific antibody complexes can be created by linkage of two or more immunoglobulins of different specificity for tumor antigens and/or effector cell antigens, either at the peptide or nucleic acid level.

Immunoglobulin can be produced *in vivo* in human or non-human species, or *in vitro* from immunoglobulin encoding DNA or cDNA isolated from libraries of DNA (e.g., phage display libraries). Immunoglobulin can also be modified genetically or chemically to incorporate human polypeptide sequences into non-human coding sequences (commonly referred to as humanization). Additionally, immunoglobulins can be modified chemically or genetically to incorporate protein, lipid, or carbohydrate moieties. Potential modifications could also include naturally occurring or synthetic molecular entities that are either directly toxic for tumor cells or serve as ligands or receptors for biologically active molecules that

could suppress tumor growth. For example, growth factors, cytokines, chemokines and their respective receptors, immunologically active ligands or receptors, hormones or naturally occurring or synthetic toxins all represent biologically active molecules that could interact with suitably modified immunoglobulins and their targets.

As used herein, an "anti-cancer antibody or fragment thereof is an antibody or an antibody fragment that binds to a cancer or tumor antigen.

Some commercially available anti-cancer antibodies along with their commercial source are as follows: anti-CD20 mAb (monoclonal antibody), rituximab, (Rituxan™, IDEC-Y2Bf), Rituxan™, Non-Hodgkin's lymphoma, B cell lymphoma (IDEC/Genentech); anti-CD20 mAb, tositumomab Bexxar, Non-Hodgkin's lymphoma (Corixa/GlaxoSmithKline); anti-HER2, trastuzumab, Herceptin™, breast and ovarian cancer (Genentech); anti-HER2, MDX-210, prostate, non-small cell lung cancer, breast, pancreatic, ovarian, renal and colon cancer (Medarex/Novartis); anti-CA125 mAb, oregovomab, B43.13, Ovarex™, ovarian cancer (Altarex); Breva-Rex, multiple myeloma, breast, lung, ovarian (Altarex); AR54, ovarian, breast, lung (Altarex); GivaRex, pancreas, stomach, colorectal (Altarex); ProstaRex, prostate (Altarex); anti-EGF receptor mAb, IMC-C225, Erbitux™, breast, head and neck, non-small cell lung, renal, prostate, colorectal and pancreatic cancer (ImClone Systems); anti-EGF receptor mAb, MDX-447, head and neck, prostate, lung, bladder, cervical, ovarian cancer (Medarex/Merck); gemtuzumab ozogamicin, Mylotarg, CMA-676, anti-CD33 (Wyeth Pharmaceuticals); anti-tissue factor protein (TF), (Sunol); ior-c5, colorectal cancer; ceal, colorectal cancer; c5, colorectal cancer; anti-EGF receptor mAb, MDX-447, head and neck, prostate, lung, bladder, cervical and ovarian cancer (Medarex/Merck); anti-17-lA mAb, edrecolomab, Panorex, colorectal, pancreatic, lung, breast and ovarian cancer (Centocor/Glaxo/Ajinomoto); anti-CD20 mAb (Y-90 labeled), ibritumomab tiuxetan (IDEC-Y2B8), Zevalin, Non-Hodgkin's lymphoma (IDEC); anti-idiotypic mAb mimic of ganglioside GD3 epitope, BEC2, small cell lung carcinoma, melanoma (ImClone Systems); anti-HLA-DrIO mAb (131 1LYM-I), Oncolym™, Non-Hodgkin's lymphoma (Peregrine Pharmaceuticals); anti-CD33 humanized mAb (SMART M195), Zamyl™, acute myeloid leukemia, acute promyelocytic leukemia (Protein Design Labs); anti-CD52 humAb (LDP-03), CAMPATH, chronic lymphocytic leukemia (Millenium Pharmaceuticals/Ilex Oncology); anti-CDl mAb, ior t6, cancer (Center of Molecular Immunology); anti-CAR (complement activating receptor) mAb, MDX-1 1, myeloid leukemia (Medarex); humanized bispecific mAb conjugates (complement cascade activators), MDX-22, myeloid leukemia (Medarex); OV 103

(Y-90 labeled antibody), celogovab, OncoScint™, ovarian and prostate cancer (Cytogen); anti-17-lAmAb, 3622W94, non-small cell lung carcinoma, prostate cancer (Glaxo Wellcome pic); anti-VEGF (RhumAb-VEGF), bevacizumab, Avastin™, lung, breast, prostate, renal and colorectal cancer (Genentech); anti-TAC (IL-2 receptor) humanized Ab (SMART), daclizumab, Zenapax, leukemia, lymphoma (Protein Design Labs); anti-TAG-72 partially humanized bispecific Ab, MDX-220, lung, colon, prostate, ovarian, endometrial, pancreatic and gastric cancer (Medarex); anti-idiotypic mAb mimic of high molecular weight proteoglycan (I-Mel-1), MELIMMUNE-I, melanoma (IDEC); anti-idiotypic mAb mimic of high molecular weight proteoglycan (I-Mel-2), MELIMMUNE-2, melanoma (IDEC); anti-CEA Ab (hMN14), CEACide™, colorectal cancer and other cancers (Immunomedics); Pretarget™ radioactive targeting agents, cancer (NeoRx); hmAbHl 1 scFv fragment (NovomAb-G2), HIl scFv, cancer (Viventia Biotech); anti-DNA or DNA-associated proteins (histones) mAb and conjugates, TNT (e.g. Cotara™), cancer (Peregrine Pharmaceuticals); Gliomab-H mAb, brain cancer, melanoma, neuroblastoma (Viventia Biotech); GNI-250 mAb, colorectal cancer (Wyeth); anti-EGF receptor mAb, EMD-72000, cancer (Merck KgaA); anti-CD22 humanized Ab, LymphoCide, Non-Hodgkin's lymphoma (Immunomedics); anti-CD33 mAb conjugate with calicheamicin (CMA 676), gemtuzumab ozogamicin, Mylotarg™, acute myelogenous leukemia (Wyeth); Monopharm-C, colon, lung and pancreatic cancer (Viventia Biotech); anti-idiotypic human mAb to GD2 ganglioside, 4B5, melanoma, small-cell lung cancer, neuroblastoma (Viventia Biotech); anti-EGF receptor humanized Ab, ior egf/r3, cancers of epithelial origin (Center of Molecular Immunology); anti-ior c2 glycoprotein mAb, ior c5, colorectal and ovarian cancer (Center of Molecular Immunology); BABS (biosynthetic antibody binding site) proteins, breast cancer (Chiron); anti-FLK-2/FLT-3 mAb, cancer (tumor-associated angiogenesis) (ImClone Systems); mAb/small-molecule conjugate, TAP (tumor-activated prodrug), cancer (ImmunoGen); anti-GD-2 bispecific mAb, MDX-260, melanoma, glioma, neuroblastoma (Medarex); antinuclear autoantibodies (binds nucleosomes), ANA Ab, cancer (Procyon Biopharma); anti-HLA-DR Ab (SMART IDlO Ab), Remitogen™, Non-Hodgkin's B-cell lymphoma (Protein Design Labs); SMART ABL 364 Ab, epithelial cell cancers, breast, lung and colon cancer (Protein Design Labs/Novartis); anti-CEA 1131-labeled mAb, ImmuRAIT-CEA, colorectal cancer (Immunomedics).

The antibody or antibody fragment provided herein can be used additionally for delivery of toxic substances to cancer cells. They may be conjugated to a toxin (for example derived from a plant, fungus, or bacteria), a chemotherapeutic agent or a radioisotope.

The toxin may be selected from the group consisting of A chain toxin, deglycosylated A chain toxin, ribosome inactivating protein, α-sarcin, aspergillin, restrictocin, ribonuclease, diptheria toxin, Pseudomonas exotoxin, maytansinoids and ricin (e.g., from castor beans), or maybe a member of members of the enediyne family of molecules, such as calicheamicin and esperamicin but is not so limited.

Chemotherapeutic agents that may be conjugated to the antibody or antibody fragment may be selected from the group consisting of an anti-metabolite, an anthracycline, a vinca alkaloid, an antibiotic, an alkylating agent, and an epipodophyllotoxin, but is not so limited. Examples of such chemotherapeutic agents include but are not limited to methotrexate, doxorubicin, melphalan, chlorambucil, ARA-C, vindesine, mitomycin C, cis-platinum, etoposide, bleomycin and 5-fluorouaracil.

Examples of radioisotopes that may be conjugated to the antibody or antibody fragment include alpha-emitting isotopes, beta-emitting isotopes, and isotopes that emit low energy electrons. Examples of alpha-emitting include ^{225}Ac, ^{211}At, ^{212}Bi, or ^{213}Bi. Examples of beta-emitting isotopes include ^{186}Rh, ^{188}Rh, ^{177}Lu, ^{90}Y, ^{131}I or ^{67}Cu. Examples of isotopes that emit low energy electrons include ^{125}I, ^{123}I or ^{77}Br.

Combinations of the various toxins could also be coupled to one or more antibody molecule thereby accommodating variable cytotoxicity.

The coupling of one or more toxin, chemotherapeutic agent, and/or radioisotope, to the antibody is envisioned to include many chemical mechanisms, for instance covalent binding, affinity binding, intercalation, coordinate binding, and complexation all of which are standard protocols known in the art.

The covalent binding can be achieved either by direct condensation of existing side chains or by the incorporation of external bridging molecules. Many bivalent or polyvalent agents are useful in coupling protein molecules to other proteins, peptides or amine functions, etc. For example, the literature is replete with coupling agents such as carbodiimides, diisocyanates, glutaraldehyde, diazobenzenes, and hexamethylene diamines. This list is not intended to be exhaustive of the various coupling agents known in the art but, rather, is exemplary of the more common coupling agents.

In some embodiments, it is contemplated that one may wish to first derivatize the antibody, and then attach the toxin component to the derivatized product. Suitable cross-linking agents for use in this manner include, for example, SPDP (N-succinimidyl-3-(2-

pyridyldithio)propionate), and SMPT, 4-succinimidyl-oxycarbonyl-methyl-(2-pyridyldithio)toluene.

In addition, protein toxins can be fused to the antibody by genetic methods to form a hybrid immunotoxin fusion protein. To make a fusion immunotoxin protein a nucleic acid molecule is generated that encodes the antibody, a fragment of the antibody, a single chain antibody, or a subunit of an antibody linked to a protein toxin. Such fusion proteins contain at least a targeting agent (e.g., the antibody subunit) and a toxin, operatively attached. The fusion proteins may also include additional peptide sequences, such as peptide spacers which operatively attach the targeting agent and toxin compound, as long as such additional sequences do not appreciably affect the targeting or toxin activities of the fusion protein. The two proteins can be attached by a peptide linker or spacer, such as a glycine-serine spacer peptide, or a peptide hinge, as is well known in the art. Thus, for example, the C-terminus of the antibody can be fused to the N-terminus of the protein toxin molecule to form an immunotoxin that retains the binding properties of the antibody. Other fusion arrangements will be known to one of ordinary skill in the art.

To express the fusion immunotoxin, the nucleic acid encoding the fusion protein is inserted into an expression vector in accordance with standard methods, for stable expression of the fusion protein, preferably in mammalian cells, such as CHO cells. The fusion protein can be isolated and purified from the cells or culture supernatant using standard methodology, such as a an antibody affinity column.

Radionuclides typically are coupled to an antibody by chelation. For example, in the case of metallic radionuclides, a bifunctional chelator is commonly used to link the isotope to the antibody or other protein of interest. Typically, the chelator is first attached to the antibody, and the chelator-antibody conjugate is contacted with the metallic radioisotope. A number of bifunctional chelators have been developed for this purpose, including the diethylenetriamine pentaacetic acid (DTPA) series of amino acids described in U.S. patents 5,124,471, 5,286,850 and 5,434,287, which are incorporated herein by reference. As another example, hydroxamic acid-based bifunctional chelating agents are described in U.S. patent 5,756,825, the contents of which are incorporated herein. Another example is the chelating agent termed /?-SCN-Bz-HEHA (1,4,7,10,13,16-hexaazacyclo-octadecane-N,N',N'',N''',N'''',N'''''-hexaacetic acid) (Deal et al., *J. Med. Chem.* 42:2988, 1999), which is an effective chelator of radiometals such as ^{225}Ac.

Antibodies to cancer antigens, vasculature, and microbial antigens can be modified in this manner. Antibodies to vasculature are particularly important because, generally, solid tumors are dependent upon newly formed blood vessels to survive. As a result, one strategy of many cancer medicaments is to attack the blood vessels feeding a tumor and/or the connective tissues (or stroma) supporting such blood vessels.

The invention embraces a number of classes of antibodies and fragments thereof including but not limited to antibodies directed to cancer antigens (as described above), cell surface molecule, stromal cell molecules, extracellular matrix molecules, and tumor vasculature associated molecules.

A cell surface molecule is a molecule that is expressed at the surface of a cell. In addition to an extracellular domain, it may further comprise a transmembrane domain and a cytoplasmic domain. Examples include HER 2, CD20, CD33, EGF receptor, HLA markers such as HLA-DR, CD52, CDl, CEA, CD22, GD2 ganglioside, FLK2/FLT3, VEGF, VEGFR, and the like.

A stromal cell molecule is a molecule expressed by a stromal cell. Examples include but are not limited to FAP and DPP-IV.

An extracellular matrix molecule is a molecule found in the extracellular matrix. Examples include but are not limited to collagen, glycosaminoglycans (GAGs), proteoglycans, elastin, fibronectin and laminin.

A tumor vasculature associated molecule is a molecule expressed by vasculature of a tumor (i.e., a solid cancer rather than a systemic cancer such as leukemia). As with a cancer antigen, a tumor vasculature associated molecule may be expressed by normal vasculature however its presence on vasculature of a tumor makes it a suitable target for anti-cancer therapy. In some instances, the tumor vasculature associated molecule is expressed at a higher level in tumor vasculature than it is in normal vasculature. Examples include but are not limited to endoglin (see U.S. Pat. No. 5,660,827), ELAM-I, VCAM-I, ICAM-I, ligand reactive with LAM-I, MHC class II antigens, aminophospholipids such as phosphatidylserine and phosphatidylethanolamine (as described in U.S. Pat. No. 6,312,694), VEGFRl (FIt-I) and VEGFR2 (KDR/Flk-1), and other tumor vasculature associated antigens such as those described in U.S. Pat. No. 5,776,427. Antibodies to endoglin are described in U.S. Pat. No. 5,660,827 and include TEC-4 and TEC-1 1, and antibodies that recognize identical epitopes to these antibodies. Antibodies to aminophospholipids are described in U.S. Pat. No. 6,312,694. Antibodies that inhibit VEGF are described in U.S. Pat. No. 6,342,219 and include 2C3

(ATCC PTA 1595). Other antibodies that are specific for tumor vasculature include antibodies that react to a complex of a growth factor and its receptor such as a complex of FGF and the FGFR or a complex of TGFβ and the TGFβR. Antibodies of this latter class are described in U.S. Pat. No. 5,965,132, and include GV39 and GV97.

In some preferred embodiments of the invention, -the antibody is rituximab (RITUXAN), bevacizumab (AVASTIN), cetuximab (ERBITUX), trastuzumab (HERCEPTIN), tositumomab (BEXXAR), or alemtuzumab (CAMPATH). mitumomab (BEC2), CeaVac, centuximab (IMC-C225), epratuzumab (LYMPHOCIDE), MDX-210, gemtuzumab ozogamicin (MYLOTARG), edrecolomab (PANOREX), pemtumomab (THERAGYN), Zamyl, and ibritumomab tituxetan (ZEVALIN). The invention also covers antibody fragments thereof.

The antibody or antibody fragment may be specific for CD20. Examples of such antibodies include rituximab (RITUXAN), ibritumomab tiuxetan (ZEVALIN), and tositumomab (BEXXAR). The antibody or antibody fragment may be specific for CD22. Examples include epratuzumab (LYMPHOCIDE) and MDX-210. The antibody or antibody fragment may be specific for EGF receptor. Examples include cetuximab (ERBITUX, IMC-C225), trastuzumab (HERCEPTIN), MDX-210, MDX-447, EMD-72000, and IOR EGF/R3. The antibody or antibody fragment maybe specific for CD33. Examples include gemtuzumab (MYLOTARG, CMA 676) and SMART M 195 (ZAMYL). The antibody or antibody fragment may be specific for CD52. Examples include alemtuzumab (CAMPATH, LDP-03). The antibody or antibody fragment may be specific for HLA-DR. Examples include lym-1 (ONCOLYM) and SMART IDlO Ab (REMITOGEN). The antibody or antibody fragment may be specific for αVβ3 integrin. Examples include VITAXIN. The antibody or antibody fragment may be specific for TAG. The antibody or antibody fragment may be specific for CDl . Examples include I0R-T6.

Other antibodies that can be used according to the invention include anti-TNFα antibody such as infliximab (Remicade) and etanercept (Enbrel) for rheumatoid arthritis and Crohn's disease palivizumab; anti-RSV antibody for pediatric subjects; bevacizumab, breast cancer; alemtuzumab, Campath-IH, breast and renal cancer, melanoma, B cell chronic lymphocytic leukemia (Millennium and ILEX); BLyS-mAb, fSLE and rheumatoid arthritis; anti-VEGF2, melanoma, breast cancer; anti-Trail receptor; B3 mAb, breast cancer; ml 70 mAb, breast cancer; mAB BR96, breast cancer; Abx-Cbl mAb, graft versus host disease.

The agents of the invention can be used with normal and hyper-immune globulin therapy. Normal immune globulin therapy utilizes an antibody product from the serum of normal blood donors. This pooled product contains low titers of antibody to a wide range of antigens such as those of infectious pathogens (e.g., bacteria, viruses such as hepatitis A, parvovirus, enterovirus, fungi and parasites). Hyper-immune globulin therapy utilizes antibodies which are prepared from the serum of individuals who have high titers of an antibody to a particular antigen. The antibodies may be those that are currently used or in development for treating infectious diseases. Examples include zoster immune globulin (useful for the prevention of varicella-zoster in immunocompromised children and neonates), human rabies immunoglobulin (useful in the post-exposure prophylaxis of a subject bitten by a rabid animal), hepatitis A or B immune globulin (useful in the prevention of hepatitis A or B virus, especially in a subject exposed to the virus), RSV immune globulin (useful in the treatment of respiratory syncitial virus infections), tetanus immunoglobulin; measles immunoglobulin (useful in the prevention of infection in immunocompromised or adult subjects); rubella immunoglobulin (useful in the prevention of infection in pregnant female subjects).

Other antibodies for infectious diseases include anti-shiga toxin antibodies, anti-staphylococcal antibodies (Virion Systems), and the like. An example of an anti-viral that is an antibody is palivizumab (SYNAGIS) which is used prophylactically for RSV infections in infants and children.

It is to be understood that the antibodies embraced by the invention include those recited explicitly herein and also those that bind to the same epitope as those recited herein.

Also useful in the invention are antibodies such as the following, all of which are commercially available:

BAX Antibodies: Anti-Human Bax Antibodies (Monoclonal), Anti-Human Bax Antibodies (Polyclonal), Anti-Murine Bax Antibodies (Monoclonal), Anti-Murine Bax Antibodies (Polyclonal);

Fas / Fas Ligand Antibodies: Anti-Human Fas / Fas Ligand Antibodies, Anti-Murine Fas / Fas Ligand Antibodies Granzyme Antibodies Granzyme B Antibodies;

BCL Antibodies: Anti Cytochrome C Antibodies, Anti-Human BCL Antibodies (Monoclonal), Anti-Human bcl Antibodies (Polyclonal), Anti-Murine bcl Antibodies (Monoclonal), Anti-Murine bcl Antibodies (Polyclonal);

Miscellaneous Apoptosis Antibodies: Anti TRADD, TRAIL, TRAFF, DR3 Antibodies Anti-Human Fas / Fas Ligand Antibodies Anti-Murine Fas / Fas Ligand Antibodies;

Miscellaneous Apoptosis Related Antibodies: BIM Antibodies: Anti Human, Murine bim Antibodies (Polyclonal), Anti-Human, Murine bim Antibodies (Monoclonal);

PARP Antibodies: Anti-Human PARP Antibodies (Monoclonal), Anti-Human PARP Antibodies (Polyclonal) Anti-Murine PARP Antibodies;

Caspase Antibodies: Anti-Human Caspase Antibodies (Monoclonal), Anti-Murine Caspase Antibodies;

Anti-CD Antibodies: Anti-CD29, PLl 8-5 PanVera, Anti-CD29, PL4-3 PanVera, Anti-CD41a, PT25-2 PanVera, Anti-CD42b, PL52-4 PanVera, Anti-CD42b, GUR20-5 PanVera, Anti-CD42b, WGA-3 PanVeraAnti-CD43, 1D4 PanVera, Anti-CD46, MCP75-6 PanVera, Anti-CD61, PLl 1-7 PanVera, Anti-CD61, PL8-5 PanVera, Anti-CD62/P-slctn, PL7-6 PanVera, Anti-CD62/P-slctn, WGA-I PanVera, Anti-CD 154, 5F3 PanVera; and anti-CD 1, anti-CD2, anti-CD3, anti-CD4, anti-CD5, anti-CD6, anti-CD7, anti-CD8, anti-CD9, anti-CDlO, anti-CDl 1, anti-CD12, anti-CD13, anti-CD14, anti-CD15, anti-CD16, anti-CD17, anti-CD18, anti-CD19, anti-CD20, anti-CD21, anti-CD22, anti-CD23, anti-CD24, anti-CD25, anti-CD26, anti-CD27, anti-CD28, anti-CD29, anti-CD30, anti-CD31, anti-CD32, anti-CD33, anti-CD34, anti-CD35, anti-CD36, anti-CD37, anti-CD38, anti-CD39, anti-CD40 anti-CD41, anti-CD42, anti-CD43, anti-CD44, anti-CD45, anti-CD46, anti-CD47, anti-CD48, anti-CD49, anti-CD50, anti-CD51, anti-CD52, anti-CD53, anti-CD54, anti-CD55, anti-CD56, anti-CD57, anti-CD58, anti-CD59, anti-CD60, anti-CD61, anti-CD62, anti-CD63, anti-CD64, anti-CD65, anti-CD66, anti-CD67, anti-CD68, anti-CD69, anti-CD70, anti-CD71, anti-CD72, anti-CD73, anti-CD74, anti-CD75, anti-CD76, anti-CD77, anti-CD78, anti-CD79, anti-CD80, anti-CD81, anti-CD82, anti-CD83, anti-CD84, anti-CD85, anti-CD86, anti-CD87, anti-CD88, anti-CD89, anti-CD90, anti-CD91, anti-CD92, anti-CD93, anti-CD94, anti-CD95, anti-CD96, anti-CD97, anti-CD98, anti-CD99, anti-CDIOO, anti-CD 101, anti-CD 102, anti-CD103, anti-CD104, anti-CD 105, anti-CD 106, anti-CD 107, anti-CD 108, anti-CD 109, anti-CDl 10, anti-CDl 11, anti-CDl 12, anti-CDl 13, anti-CDl 14, anti-CDl 15, anti-CDl 16, anti-CDl 17, anti-CDl 18, anti-CDl 19, anti-CD120, anti-CD121, anti-CD122, anti-CD123, anti-CD124, anti-CD125, anti-CD126, anti-CD127, anti-CD128, anti-CD129, anti-CD130, anti-CD131, anti-CD132, anti-CD133, anti-CD134, anti-CD135, anti-CD136, anti-CD137, anti-CD138, anti-CD139, anti-CD140, anti-CD141, anti-CD142, anti-CD143, anti-CD144, anti-CD145, anti-CD146, anti-

CD147, anti-CD148, anti-CD149, anti-CD150, anti-CD151, anti-CD152, anti-CD153, anti-CD154, anti-CD155, anti-CD156, anti-CD157, anti-CD158, anti-CD159, anti-CD160, anti-CD161, anti-CD162, anti-CD163, anti-CD164, anti-CD165, anti-CD166, anti-CD167, anti-CD168, anti-CD169, anti-CD170, anti-CD171, anti-CD172, anti-CD173, anti-CD174, anti-CD175, anti-CD176, anti-CD177, anti-CD178, anti-CD179, anti-CD180, anti-CD181, anti-CD182, anti-CD183, anti-CD184, anti-CD185, anti-CD186, anti-CD187, anti-CD188, anti-CDl 89, anti-CD190, anti-CD191, anti-CD192, anti-CD193, anti-CD194, anti-CD195, anti-CD 196, anti-CD 197, anti-CD 198, anti-CD 199, anti-CD200, anti-CD201, anti-CD202, anti-CD203, anti-CD204, anti-CD205, anti-CD206, anti-CD207, anti-CD208, anti-CD209, anti-CD210, anti-CD211, anti-CD212, anti-CD213, anti-CD214, anti-CD215, anti-CD216, anti-CD217, anti-CD218, anti-CD219, anti-CD220, anti-CD221, anti-CD222, anti-CD223, anti-CD224, anti-CD225, anti-CD226, anti-CD227, anti-CD228, anti-CD229, anti-CD230, anti-CD231, anti-CD232, anti-CD233, anti-CD234, anti-CD235, anti-CD236, anti-CD237, anti-CD238, anti-CD239, anti-CD240 anti-CD241, anti-CD242, anti-CD243, anti-CD244, anti-CD245, anti-CD246, anti-CD247, anti-CD248, anti-CD249, anti-CD250, and the like.

Human Chemokine Antibodies: Human CNTF Antibodies, Human Eotaxin Antibodies, Human Epithelial Neutrophil Activating Peptide-78, Human Exodus Antibodies, Human GRO Antibodies, Human HCC-I Antibodies, Human 1-309 Antibodies, Human EP-IO Antibodies, Human I-TAC Antibodies, Human LIF Antibodies, Human Liver-Expressed Chemokine Antibodies, Human lymphotoxin Antibodies, Human MCP Antibodies, Human MIP Antibodies, Human Monokine Induced by IFN-gamma Antibodies, Human NAP-2 Antibodies, Human NP-I Antibodies, Human Platelet Factor-4 Antibodies, Human RANTES Antibodies, Human SDF Antibodies, Human TECK Antibodies;

Murine Chemokine Antibodies: Human B-CeIl Attracting Murine Chemokine Antibodies, Chemokine- 1 Antibodies, Murine Eotaxin Antibodies, Murine Exodus Antibodies, Murine GCP-2 Antibodies, Murine KC Antibodies, Murine MCP Antibodies, Murine MIP Antibodies, Murine RANTES Antibodies, Rat Chemokine Antibodies, Rat Chemokine Antibodies, Rat CNTF Antibodies, Rat GRO Antibodies, Rat MCP Antibodies, Rat MIP Antibodies, Rat RANTES Antibodies;

Cytokine / Cytokine Receptor Antibodies: Human Biotinylated Cytokine / Cytokine Receptor Antibodies, Human IFN Antibodies, Human IL Antibodies, Human Leptin Antibodies, Human Oncostatin Antibodies, Human TNF Antibodies, Human TNF Receptor Family Antibodies, Murine Biotinylated Cytokine / Cytokine Receptor Antibodies, Murine

EFN Antibodies, Murine IL Antibodies, Murine TNF Antibodies, Murine TNF Receptor Antibodies; anti-CCR4 antibody;

Rat Cytokine / Cytokine Receptor Antibodies: Rat Biotinylated Cytokine / Cytokine Receptor Antibodies, Rat IFN Antibodies, Rat IL Antibodies, Rat TNF Antibodies;

ECM Antibodies: Collagen / Procollagen, Laminin, Collagen (Human), Laminin (Human), Procollagen (Human), Vitronectin / Vitronectin Receptor, Vitronectin (Human), Vitronectin Receptor (Human), Fibronectin / Fibronectin Receptor, Fibronectin (Human), Fibronectin Receptor (Human);

Growth Factor Antibodies: Human Growth Factor Antibodies, Murine Growth Factor Antibodies, Porcine Growth Factor Antibodies;

Miscellaneous Antibodies: Baculovirus Antibodies, Cadherin Antibodies, Complement Antibodies, CIq Antibodies, VonWillebrand Factor Antibodies, Cre Antibodies, HIV Antibodies, Influenza Antibodies, Human Leptin Antibodies , Murine Leptin Antibodies, Murine CTLA-4 Antibodies, Human CTLA-4 Antibodies, P450 Antibodies, RNA Polymerase Antibodies;

Neurobio Antibodies: Amyloid Antibodies, GFAP Antibodies, Human NGF Antibodies , Human NT-3 Antibodies , Human NT-4 Antibodies.

Still other antibodies can be used in the invention and these include antibodies listed in references such as the MSRS Catalog of Primary Antibodies, and Linscott's Directory.

The invention encompasses the use of both antibodies and antibody fragments. The antibodies may be monoclonal or polyclonal, and can be prepared by conventional methodology. They may further be isolated or present in an ascites fluid. Such antibodies can be further manipulated to create chimeric or humanized antibodies as will be discussed in greater detail below.

Significantly, as is well-known in the art, only a small portion of an antibody molecule, the paratope, is involved in the binding of the antibody to its epitope (see, in general, Clark, W.R. (1986) The Experimental Foundations of Modern Immunology Wiley & Sons, Inc., New York; Roitt, I. (1991) Essential Immunology, 7th Ed., Blackwell Scientific Publications, Oxford). The pFc[1]and Fc regions, for example, are effectors of the complement cascade but are not involved in antigen binding. An antibody from which the pFc' region has been enzymatically cleaved, or which has been produced without the pFc' region, designated an $F(ab')_2$ fragment, retains both of the antigen binding sites of an intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been

produced without the Fc region, designated an Fab fragment, retains one of the antigen binding sites of an intact antibody molecule. Proceeding further, Fab fragments consist of a covalently bound antibody light chain and a portion of the antibody heavy chain denoted Fd. The Fd fragments are the major determinant of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity) and Fd fragments retain epitope-binding ability in isolation.

Within the antigen-binding portion of an antibody, as is well-known in the art, there are complementarity determining regions (CDRs), which directly interact with the epitope of the antigen, and framework regions (FRs), which maintain the tertiary structure of the paratope (see, in general, Clark, 1986; Roitt, 1991). In both the heavy chain Fd fragment and the light chain of IgG immunoglobulins, there are four framework regions (FRl through FR4) separated respectively by three complementarity determining regions (CDRl through CDR3). The CDRs, and in particular the CDR3 regions, and more particularly the heavy chain CDR3, are largely responsible for antibody specificity.

It is now well-established in the art that the non-CDR regions of a mammalian antibody may be replaced with similar regions of co-specific or heterospecific antibodies while retaining the epitopic specificity of the original antibody. This is most clearly manifested in the development and use of "humanized" antibodies in which non-human CDRs are covalently joined to human FR and/or Fc/pFc' regions to produce a functional antibody. Thus, for example, PCT International Publication Number WO 92/04381 teaches the production and use of humanized murine RSV antibodies in which at least a portion of the murine FR regions has been replaced by FR regions of human origin. Such antibodies, including fragments of intact antibodies with antigen-binding ability, are often referred to as "chimeric" antibodies. Commercial sources of humanized or chimeric antibodies include GenPharm, Xenotech, AbGenix and CellGeneSys.

Thus, as will be apparent to one of ordinary skill in the art, the present invention also provides for $F(ab')_2$, Fab, Fv and Fd fragments; chimeric antibodies in which the Fc and/or FR and/or CDRl and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric $F(ab')_2$ fragment antibodies in which the FR and/or CDRl and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric Fab fragment antibodies in which the FR and/or CDRl and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; and chimeric Fd fragment antibodies in which

the FR and/or CDRl and/or CDR2 regions have been replaced by homologous human or non-human sequences. The present invention also includes so-called single chain antibodies.

The invention also embraces the use of adjuvants. Adjuvant substances derived from microorganisms, such as bacillus Calmette-Guerin, heighten the immune response and enhance resistance to tumors in animals. Adjuvants that may be combined with the agents of the invention include alum, immunostimulatory oligonucleotides such as CpG oligonucleotides, QS-21, and the like. These and other adjuvants are listed herein in greater detail. Other therapeutic agents include but are not limited to nucleic acid adjuvants, non-nucleic acid adjuvants, cytokines, non-immunotherapeutic antibodies, antigens, etc.

A "nucleic acid adjuvant" is an adjuvant that is a nucleic acid. Examples include immunostimulatory nucleic acid molecules such as those containing CpG dinucleotides, as described in U.S. Patents US 6,194,388Bl, issued February 27, 2001, US 6,207,646 Bl, issued March 27, 2001, and US 6,239,1 16 Bl, issued May 29, 2001.

A "non-nucleic acid adjuvant" is any molecule or compound except for the immunostimulatory nucleic acids described herein which can stimulate the humoral and/or cellular immune response. Non-nucleic acid adjuvants include, for instance, adjuvants that create a depo effect, immune-stimulating adjuvants, adjuvants that create a depo effect and stimulate the immune system and mucosal adjuvants.

An "adjuvant that creates a depo effect" as used herein is an adjuvant that causes an antigen, such as a cancer antigen present in a cancer vaccine, to be slowly released in the body, thus prolonging the exposure of immune cells to the antigen. This class of adjuvants includes but is not limited to alum (e.g., aluminum hydroxide, aluminum phosphate); or emulsion-based formulations including mineral oil, non-mineral oil, water-in-oil or oil-in-water-in oil emulsion, oil-in-water emulsions such as Seppic ISA series of Montanide adjuvants (e.g., Montanide ISA 720, AirLiquide, Paris, France); MF-59 (a squalene-in-water emulsion stabilized with Span 85 and Tween 80; Chiron Corporation, Emeryville, CA; and PROVAX (an oil-in-water emulsion containing a stabilizing detergent and a micelle-forming agent; IDEC Pharmaceuticals Corporation, San Diego, CA).

An "immune stimulating adjuvant" is an adjuvant that causes activation of a cell of the immune system. It may, for instance, cause an immune cell to produce and secrete cytokines. This class of adjuvants includes but is not limited to saponins purified from the bark of the *Q. saponaria* tree, such as QS21 (a glycolipid that elutes in the 2 1st peak with HPLC fractionation; Antigenics, Inc., Waltham, MA); poly [di (carboxylatophenoxy) phosphazene

(PCPP polymer; Virus Research Institute, USA); derivatives of lipopolysaccharides such as monophosphoryl lipid A (MPL; Ribi ImmunoChem Research, Inc., Hamilton, MT), muramyl dipeptide (MDP; Ribi) and threonyl-muramyl dipeptide (t-MDP; Ribi); OM-1 74 (a glucosamine disaccharide related to lipid A; OM Pharma SA, Meyrin, Switzerland); and Leishmania elongation factor (a purified *Leishmania* protein; Corixa Corporation, Seattle, WA).

"Adjuvants that create a depo effect and stimulate the immune system" are those compounds which have both of the above- identified functions. This class of adjuvants includes but is not limited to ISCOMS (Immunostimulating complexes which contain mixed saponins, lipids and form virus-sized particles with pores that can hold antigen; CSL, Melbourne, Australia); SB-AS2 (SmithKline Beecham adjuvant system #2 which is an oil-in-water emulsion containing MPL and QS21: SmithKline Beecham Biologicals [SBB], Rixensart, Belgium); SB-AS4 (SmithKline Beecham adjuvant system #4 which contains alum and MPL; SBB, Belgium); non-ionic block copolymers that form micelles such as CRL 1005 (these contain a linear chain of hydrophobic polyoxpropylene flanked by chains of polyoxyethylene; Vaxcel, Inc., Norcross, GA); and Syntex Adjuvant Formulation (SAF, an oil-in-water emulsion containing Tween 80 and a nonionic block copolymer; Syntex Chemicals, Inc., Boulder, CO).

A "non-nucleic acid mucosal adjuvant" as used herein is an adjuvant other than an immunostimulatory nucleic acid that is capable of inducing a mucosal immune response in a subject when administered to a mucosal surface in conjunction with an antigen. Mucosal adjuvants include but are not limited to Bacterial toxins: e.g., Cholera toxin (CT), CT derivatives including but not limited to CT B subunit (CTB) (Wu et ah, 1998, Tochikubo et al., 1998); CTD53 (VaI to Asp) (Fontana et al., 1995); CTK97 (VaI to Lys) (Fontana et al., 1995); CTKl 04 (Tyr to Lys) (Fontana et al., 1995); CTD53/K63 (VaI to Asp, Ser to Lys) (Fontana et al., 1995); CTH54 (Arg to His) (Fontana et al., 1995); CTN107 (His to Asn) (Fontana et al., 1995); CTEl 14 (Ser to GIu) (Fontana et al., 1995); CTEl 12K (GIu to Lys) (Yamamoto et al., 1997a); CTS61F (Ser to Phe) (Yamamoto et al., 1997a, 1997b); CTS106 (Pro to Lys) (Douce et al., 1997, Fontana et al., 1995); andCTK63 (Ser to Lys) (Douce et al., 1997, Fontana et al., 1995), Zonula occludens toxin, zot, Escherichia coli heat-labile enterotoxin, Labile Toxin (LT), LT derivatives including but not limited to LT B subunit (LTB) (Verweij et al., 1998); LT7K (Arg to Lys) (Komase et al., 1998, Douce et al., 1995); LT61F (Ser to Phe) (Komase et al., 1998); LTl 12K (GIu to Lys) (Komase et al., 1998);

LTH 8E (GIy to GIu) (Komase et al., 1998); LT146E (Arg to GIu) (Komase et al., 1998); LT192G (Arg to GIy) (Komase et al., 1998); LTK63 (Ser to Lys) (Marchetti et al., 1998, Douce et al., 1997, 1998, Di Tommaso et al., 1996); and LTR72 (Ala to Arg) (Giuliani et al., 1998), Pertussis toxin, PT. (Lycke et al., 1992, Spangler BD, 1992, Freytag and Clemments, 1999, Roberts et al., 1995, Wilson et al., 1995) including PT-9K/129G (Roberts et al., 1995, Cropley et al., 1995); Toxin derivatives (see below) (Holmgren et al., 1993, Verweij et al., 1998, Rappuoli et al., 1995, Freytag and Clements, 1999); Lipid A derivatives (e.g., monophosphoryl lipid A, MPL) (Sasaki et al., 1998, Vancott et al., 1998; Muramyl Dipeptide (MDP) derivatives (Fukushima et al., 1996, Ogawa et al., 1989, Michalek et al., 1983, Morisaki et al., 1983); Bacterial outer membrane proteins (e.g., outer surface protein A (OspA) lipoprotein of *Borrelia burgdorferi, outer membrane protine of Neisseria meningitidis)(Maή naro* et al., 1999, Van de Verg et al., 1996); Oil-in-water emulsions (e.g., MF59) (Barchfield et al., 1999, Verschoor et al., 1999, O'Hagan, 1998); Aluminum salts (Isaka et al., 1998, 1999); and Saponins (e.g., QS21) Aquila Biopharmaceuticals, Inc., Worcester, MA) (Sasaki et al., 1998, MacNeal et al., 1998), ISCOMS, MF-59 (a squalene-in-water emulsion stabilized with Span 85 and Tween 80; Chiron Corporation, Emeryville, CA); the Seppic ISA series of Montanide adjuvants (e.g., Montanide ISA 720; AirLiquide, Paris, France); PROVAX (an oil-in-water emulsion containing a stabilizing detergent and a micell-forming agent; IDEC Pharmaceuticals Corporation, San Diego, CA); Syntext Adjuvant Formulation (SAF; Syntex Chemicals, Inc., Boulder, CO); poly[di(carboxylatophenoxy)phosphazene (PCPP polymer; Virus Research Institute, USA) and Leishmania elongation factor (Corixa Corporation, Seattle, WA).

Cytokines and chemokines can potentially be cleaved and thereby inactivated by post proline cleaving enzymes. Administration of agents of the invention with cytokines and/or chemokines can enhance the efficacy of these latter agents by protecting them from degradation.

Immune responses can also be induced or augmented by the co-administration or co-linear expression of cytokines or chemokines (Bueler & Mulligan, 1996; Chow *et al.*, 1997; Geissler *et al.*, 1997; Iwasaki *et al.*, 1997; Kim *et al*, 1997) or B-7 co-stimulatory molecules (Iwasaki *et al.*, 1997; Tsuji *et al.*, 1997) with the agents of the invention and anti-cancer antibodies. The cytokines and/or chemokines can be administered directly or may be administered in the form of a nucleic acid vector that encodes the cytokine, such that the cytokine can be expressed *in vivo.* In one embodiment, the cytokine or chemokine is

administered in the form of a plasmid expression vector. The term "cytokine" is used as a generic name for a diverse group of soluble proteins and peptides which act as humoral regulators at nano- to picomolar concentrations and which, either under normal or pathological conditions, modulate the functional activities of individual cells and tissues. These proteins also mediate interactions between cells directly and regulate processes taking place in the extracellular environment. Cytokines also are central in directing the T cell response.

Examples of cytokines include, but are not limited to EL-I, IL-2, IL-4, IL-5, IL-6, IL-7, IL-IO, IL-12, IL-15, IL-18, granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), interferon-γ (EFN-γ), IFN-α, tumor necrosis factor (TNF), TGF-β, FLT-3 ligand, and CD40 ligand. In some embodiments, the cytokine is a ThI cytokine. In still other embodiments, the cytokine is a Th2 cytokine.

The term "chemokine" is used as a generic name for peptides or polypeptides that act principally to chemoattract effector cells of both innate and adaptive immunity. Chemokines are thought to coordinate immunological defenses against tumors and infectious agents by concentrating neutrophils, macrophages, eosinophils and T and B lymphocytes at the anatomical site in which the tumor or infectious agent is present. In addition, many chemokines are known to activate the effector cells so that their immune functions (e.g., cytolysis of tumor cells) are enhanced on a per cell basis. Two groups of chemokines are distinguished according to the positions of the first two cysteine residues that are conserved in the amino-terminal portions of the polypeptides. The residues can either be adjacent or separated by one amino acid, thereby defining the CC and CXC cytokines respectively. The activity of each chemokine is restricted to particular effector cells, and this specificity results from a cognate interaction between the chemokine and a specific cell membrane receptor expressed by the effector cells. For example, the CXC chemokines IL-8, Groα/β and ENA 78 act specifically on neutrophils, whereas the CC chemokines RANTES, MIP-I α and MCP-3 act on monocytes and activated T cells. In addition, the CXC chemokine IP-10 appears to have anti-angiogenic activity against tumors as well as being a chemoattractant for activated T cells. MIP-1α also reportedly has effects on hemopoietic precursor cells.

The agents of the invention are useful in non-cancer methods as well. For example, the agents can be used to prevent or treat infectious diseases such as bacterial, viral, fungal, parasitic and mycobacterial infections. An "infection" or "infectious disease", as used herein, refers to a disorder arising from the invasion of a host, superficially, locally, or systemically,

by an infectious organism. Infectious organisms include bacteria, viruses, parasites, fungi, and protozoa.

The invention also contemplates the use of the agents of the invention together with antigens such as microbial agents. Microbial antigens that can be used in the methods of the invention include whole bacteria, whole virus, whole fungi, whole parasites, and fragments thereof. Examples include non-infectious human papillomavirus-like particles (VLP) (which can be used as a cancer antigen as well, particularly for cervical cancer); and the like.

The agents can also be used prophylactically to prevent infection during periods of heightened risk, including for example flu season, epidemics, and travel to places where the risk of pathogen exposure is high. Many of the cytokines and chemokines induced by agents can prime a subject and prepare it for passive exposure to a pathogen. The rate at which agents of the invention stimulate these cytokines and chemokines (e.g., IL-8) is useful particularly where pathogen exposure cannot be anticipated.

Subjects having an infectious disease are those that exhibit symptoms of infectious disease (e.g., rapid onset, fever, chills, myalgia, photophobia, pharyngitis, acute lymphadenopathy, splenomegaly, gastrointestinal upset, leukocytosis or leukopenia) and in whom infectious pathogens or byproducts thereof can be detected. Tests for diagnosing infectious diseases are known in the art and the ordinary medical practitioner will be familiar with these laboratory tests which include but are not limited to microscopic analyses, cultivation dependent tests (such as cultures), and nucleic acid detection tests. These include wet mounts, stain-enhanced microscopy, immune microscopy (e.g., FISH), hybridization microscopy, particle agglutination, enzyme-linked immunosorbent assays, urine screening tests, DNA probe hybridization, serologic tests, etc. The medical practitioner will generally also take a full history and conduct a complete physical examination in addition to running the laboratory tests listed above.

A subject at risk of developing an infectious disease is one that is at risk of exposure to an infectious pathogen. Such subjects include those that live in an area where such pathogens are known to exist and where such infections are common. These subjects also include those that engage in high risk activities such as sharing of needles, engaging in unprotected sexual activity, routine contact with infected samples of subjects (e.g., medical practitioners), people who have undergone surgery, including but not limited to abdominal surgery, etc.

Bacteria include gram-negative and gram-positive bacteria. Examples of gram-positive bacteria include Pasteurella species, Staphylococcus species including Staphylococcus aureus,

Streptococcus species including Streptococcus pyogenes group A, Streptococcus viridans group, Streptococcus agalactiae group B, Streptococcus bovis, Streptococcus anaerobic species, Streptococcus pneumoniae, and Streptococcus faecalis, Bacillus species including Bacillus anthracis (anthrax), Corynebacterium species including Corynebacterium diphtheriae, aerobic Corynebacterium species, and anaerobic Corynebacterium species, Diphtheroids species, Listeria species including Listeria monocytogenes, Erysipelothrix species including Erysipelothrix rhusiopathiae, Clostridium species including Clostridium perfringens, Clostridium tetani, and Clostridium difficile.

Gram-negative bacteria include Neisseria species including Neisseria gonorrhoeae and Neisseria meningitidis, Branhamella species including Branhamella catarrhalis, Escherichia species including Escherichia coli, Enterobacter species, Proteus species including Proteus mirabilis, Pseudomonas species including Pseudomonas aeruginosa, Pseudomonas mallei, and Pseudomonas pseudomallei, Klebsiella species including Klebsiella pneumoniae, Salmonella species, Shigella species, Serratia species, Acinetobacter species; Haemophilus species including Haemophilus influenzae and Haemophilus ducreyi, Brucella species, Yersinia species including Yersinia pestis and Yersinia enterocolitica, Francisella species including Francisella tularensis, Pasturella species including Pasteurella multocida, Vibrio cholerae, Flavobacterium species, meningosepticum, Campylobacter species including Campylobacter jejuni, Bacteroides species (oral, pharyngeal) including Bacteroides fragilis, Fusobacterium species including Fusobacterium nucleatum, Calymmatobacterium granulomatis, Streptobacillus species including Streptobacillus moniliformis, Legionella species including Legionella pneumophila.

Other types of bacteria include acid-fast bacilli, spirochetes, and actinomycetes. Examples of acid-fast bacilli include Mycobacterium species including Mycobacterium tuberculosis and Mycobacterium leprae. Examples of spirochetes include Treponema species including Treponema pallidum, Treponema pertenue, Borrelia species including Borrelia burgdorferi (Lyme disease), and Borrelia recurrentis, and Leptospira species.

Examples of actinomycetes include Actinomyces species including Actinomyces israelii, and Nocardia species including Nocardia asteroides.

Examples of viruses include HDTV-III, LAVE, HTLV-III/LAV, HIV-III, HIV-LP, human papilloma virus infection, Cytomegaloviruses (CMV), Picornaviruses, polio viruses, hepatitis A virus, Epstein Barr virus, enteroviruses, human Coxsackie viruses, rhinoviruses, echoviruses, Calciviruses, Togaviruses, equine encephalitis viruses, rubella viruses,

Flaviruses, dengue viruses, encephalitis viruses, yellow fever viruses, Coronaviruses, Rhabdoviruses, vesicular stomatitis viruses, rabies viruses, Filoviruses, ebola virus, Paramyxoviruses, influenza viruses, parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus (RSV), Orthomyxoviruses, Bungaviruses, Hantaan viruses, phleboviruses and Nairo viruses, Arena viruses, hemorrhagic fever viruses, reoviruses, orbiviruses, rotaviruses, Birnaviruses, Hepadnaviruses, Hepatitis B virus, parvoviruses, Papovaviridae, papilloma viruses, polyoma viruses, Adenoviruses, Herpesviruses including herpes simplex virus 1 and 2, varicella zoster virus, Poxviruses, variola viruses, vaccinia viruses, Irido viruses, African swine fever virus, delta hepatitis virus, non-A, non-B hepatitis virus, Hepatitis C, Norwalk viruses, astroviruses, small pox infection, monkey pox infection, SARS infection, and unclassified viruses. In some embodiments, the methods are not intended to treat or prevent HIV infection.

Examples of fungal infections include candidiasis infection, ringworm, histoplasmosis infection, blastomycosis infections, paracoccidioidomycosis infections, cryptococcosis infections, aspergillosis infections, chromomycosis infections, mycetoma infections, pseudallescheriasis infection, and tinea versicolor infection.

Examples of parasite infections include both protozoan infections and nematode infections. These include amebiasis, Trypanosoma cruzi infection (i.e., Chagas' disease), Fascioliasis (e.g., Facioloa hepatica infection), Leishmaniasis, Plasmodium infections (e.g., malaria causing Plasmodium species infections, e.g., P. falciparum, P. knowlesi, P. malariae,) Onchocerciasis, Paragonimiasis, Trypanosoma brucei infection (i.e., Sleeping sickness), Pneumocystis infection (e.g., Pneumocystis carinii infection), Trichomonas vaginalis infection, Taenia infections, Hymenolepsis infections (e.g., Hymenolepsis nana infection), Echinococcus infections, Schistosomiasis (e.g., Schistosoma mansoni infection), neurocysticercosis, Necator americanus infection, and Trichuris trichuria infections.

Other infections that can be treated according to the methods of the invention include Chlamydia infection, mycobacterial infection such as tuberculosis and leprosy, and Rickettsiae.

The foregoing lists of infections are not intended to be exhaustive but rather exemplary. Those of ordinary skill in the art will identify other infections that are amenable to prevention and treatment using the methods of the invention.

In still other embodiments, the agents of the invention are used to stimulate immune responses against biowarfare agents such as the CDC categorized agents and where applicable the biological source of such agents. These are listed below.

CDC Category A agents include Bacillus anthracis (otherwise known as anthrax), Clostridium botulinum and its toxin (causative agent for botulism), Yersinia pestis (causative agent for the plague), variola major (causative agent for small pox), Francisella tularensis (causative agent for tularemia), and viral hemorrhagic fever causing agents such as filoviruses Ebola and Marburg and arenaviruses such as Lassa , Machυpo and Junin.

CDC Category B agents include Brucellosis (Brucella species), epsilon toxin of Clostridium perfringens, food safety threats such as Salmonella species, E. coli and Shigella, Glanders (Burkholderia mallei), Melioidosis (Burkholderia pseudomallei), Psittacosis (Chlamydia psittaci), Q fever (Coxiella burnetii), ricin toxin (from Ricinus communis —castor beans), Staphylococcal enterotoxin B, Typhus fever (Rickettsia prowazekii), viral encephalitis (alphaviruses, e.g., Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis), and water safety threats such as e.g., Vibrio cholerae, Cryptosporidium parvum.

CDC Category C agents include emerging infectious diseases such as Nipah virus and hantavirus.

In some embodiment, the agents can be used either alone or in combination with other therapeutic agents to treat chronic viral infections, particularly chronic hepatitis C infection. Currently, most but not all hepatitis C subjects are administered IFNα. Subjects who are also HIV positive fair worse with this treatment. It is believed that hepatitis C infected subjects, and especially those subjects resistant or non-responsive to IFNα treatment, may be treated using the agents. In some instances, the agents of the invention may be administered with IFNα and optionally with ribavirin also. In these subjects, the agents may also be used together with other small molecule drugs that are currently being tested for hepatitis C infection.

The agents may also be suitable for treatment of hepatitis B infection. In this latter indication, the agents may be used alone or together with EFN as well as various small molecule drugs being developed, such as IFNα-2b, acyclovir, lobucavir, ganciclovir, L-deoxythymidine, clevudine, a therapeutic vaccine, phosphonoformate (PFA), ribavirin (RBV) and thymosin alpha-1; and nucleotide and nucleoside analogues such as 2 3-dideoxy-3-

fluoroguanosine (FLG), famciclovir, lamivudine, adefovir dipivoxil, entecavir, and emtricitabine. The agents may also be used with hepatitis B-specific immunoglobulin.

The agents may also be used with lamivudine as lamivudine is reportedly associated with drug resistance. The combined use of the agents with lamivudine may reduce or eliminate the risk of drug resistance. The agents may be used in subjects already treated with lamivudine who have demonstrated drug resistance. These latter aspects of the invention apply equally to other indications for which drug resistance has been observed or is suspected. Other bacteria that have been associated with drug resistance include Staphylococcus aureus (resistance to penicillin), Streptococcus pneumoniae (resistance to penicillin), gonorrhea (resistance to penicillin), and Enterococcus faecium (penicillin). In other instances, it may be desirable to use the agents over standard drug therapy if the drug therapy is not particularly suited to a subject or induces intolerable side effects in a patient specific manner.

The agents may also be used in the treatment of tuberculosis, either alone (i.e., as a substitute for currently available drug treatments such as antibiotic therapy), or in combination with those antibiotics.

The invention contemplates the use of the agents of the invention together with anti¬microbial agents. In some instances, the agents are used together with anti-microbial agents order to reduce the risk of drug resistance by the microbial species, or for treatment following incidence of drug resistance. Examples of anti-microbial agents include anti-bacterial agents, anti-mycobacterial agents, anti-viral agents, anti-fungal agents or anti-protozoal agents.

Phrases such as "anti-microbial agent", "anti-bacterial agent", "anti-mycobacterial agent" "anti-viral agent", "anti-fungal agent", "anti-parasitic agent" and "parasiticide" have well-established meanings to those of ordinary skill in the art and are defined in standard medical texts. Briefly, anti-bacterial agents kill or inhibit the growth or function of bacteria. Anti-bacterial agents include antibiotics as well as other synthetic or natural compounds having similar functions. Antibiotics, typically, are low molecular weight molecules which are produced as secondary metabolites by cells, such as microorganisms. In general, antibiotics interfere with one or more bacterial functions or structures which are specific for the microorganism and which are not present in host cells.

A large class of anti-bacterial agents is antibiotics. Antibiotics that are effective for killing or inhibiting a wide range of bacteria are referred to as broad spectrum antibiotics. Other types of antibiotics are predominantly effective against the bacteria of the class gram-positive or gram-negative. These types of antibiotics are referred to as narrow spectrum

antibiotics. Other antibiotics which are effective against a single organism or disease and not against other types of bacteria, are referred to as limited spectrum antibiotics. Anti-bacterial agents are sometimes classified based on their primary mode of action. In general, anti¬ bacterial agents are cell wall synthesis inhibitors, cell membrane inhibitors, protein synthesis inhibitors, nucleic acid synthesis or functional inhibitors, and competitive inhibitors.

Examples of anti-bacterials include β-lactam antibiotics, penicillins (such as natural penicillins, aminopenicillins, penicillinase-resistant penicillins, carboxy penicillins, ureido penicillins), cephalosporins (first generation, second generation, and third generation cephalosporins), and other β-lactams (such as imipenem, monobactams,), β-lactamase inhibitors, vancomycin, aminoglycosides and spectinomycin, tetracyclines, chloramphenicol, erythromycin, lincomycin, clindamycin, rifampin, metronidazole, polymyxins, sulfonamides and trimethoprim, and quinolines.

Anti-bacterials include Acedapsone; Acetosulfone Sodium; Alamecin; Alexidine; Amdinocillin; Amdinocillin Pivoxil; Amicycline; Amifloxacin; Amifloxacin Mesylate; Amikacin; Amikacin Sulfate; Aminosalicylic acid; Aminosalicylate sodium; Amoxicillin; Amphomycin; Ampicillin; Ampicillin Sodium; Apalcillin Sodium; Apramycin; Aspartocin; Astromicin Sulfate; Avilamycin; Avoparcin; Azithromycin; Azlocillin; Azlocillin Sodium; Bacampicillin Hydrochloride; Bacitracin; Bacitracin Methylene Disalicylate; Bacitracin Zinc; Bambermycins; Benzoylpas Calcium; Berythromycin; Betamicin Sulfate; Biapenem; Biniramycin; Biphenamine Hydrochloride; Bispyrithione Magsulfex; Butikacin; Butirosin Sulfate; Capreomycin Sulfate; Carbadox; Carbenicillin Disodium; Carbenicillin Indanyl Sodium; Carbenicillin Phenyl Sodium; Carbenicillin Potassium; Carumonam Sodium; Cefaclor; Cefadroxil; Cefamandole; Cefamandole Nafate; Cefamandole Sodium; Cefaparole; Cefatrizine; Cefazaflur Sodium; Cefazolin; Cefazolin Sodium; Cefbuperazone; Cefdinir; Cefepime; Cefepime Hydrochloride; Cefetecol; Cefixime; Cefmenoxime Hydrochloride; Cefmetazole; Cefmetazole Sodium; Cefonicid Monosodium; Cefonicid Sodium; Cefoperazone Sodium; Ceforanide; Cefotaxime Sodium; Cefotetan; Cefotetan Disodium; Cefotiam Hydrochloride; Cefoxitin; Cefoxitin Sodium; Cefpimizole; Cefpimizole Sodium; Cefpiramide; Cefpiramide Sodium; Cefpirome Sulfate; Cefpodoxime Proxetil; Cefprozil; Cefroxadine; Cefsulodin Sodium; Ceftazidime; Ceftibuten; Ceflizoxime Sodium; Ceftriaxone Sodium; Cefuroxime; Cefuroxime Axetil; Cefuroxime Pivoxetil; Cefuroxime Sodium; Cephacetrile Sodium; Cephalexin; Cephalexin Hydrochloride; Cephaloglycin; Cephaloridine; Cephalothin Sodium; Cephapirin Sodium; Cephradine; Cetocycline Hydrochloride;

Cetophenicol; Chloramphenicol; Chloramphenicol Palmitate; Chloramphenicol Pantothenate Complex; Chloramphenicol Sodium Succinate; Chlorhexidine Phosphanilate; Chloroxylenol; Chlortetracycline Bisulfate; Chlortetracycline Hydrochloride; Cinoxacin; Ciprofloxacin; Ciprofloxacin Hydrochloride; Cirolemycin; Clarithromycin; Clinafloxacin Hydrochloride; Clindamycin; Clindamycin Hydrochloride; Clindamycin Palmitate Hydrochloride; Clindamycin Phosphate; Clofazimine; Cloxacillin Benzathine; Cloxacillin Sodium; Cloxyquin; Colistimethate Sodium; Colistin Sulfate; Coumermycin; Coumermycin Sodium; Cyclacillin; Cycloserine; Dalfopristin; Dapsone; Daptomycin; Demeclocycline; Demeclocycline Hydrochloride; Demecycline; Denofungin; Diaveridine; Dicloxacillin; Dicloxacillin Sodium; Dihydrostreptomycin Sulfate; Dipyrithione; Dirithromycin; Doxycycline; Doxycycline Calcium; Doxycycline Fosfatex; Doxycycline Hyclate; Droxacin Sodium; Enoxacin; Epicillin; Epitetracycline Hydrochloride; Erythromycin; Erythromycin Acistrate; Erythromycin Estolate; Erythromycin Ethylsuccinate; Erythromycin Gluceptate; Erythromycin Lactobionate; Erythromycin Propionate; Erythromycin Stearate; Ethambutol Hydrochloride; Ethionamide; Fleroxacin; Floxacillin; Fludalanine; Flumequine; Fosfomycin; Fosfomycin Tromethamine; Fumoxicillin; Furazolium Chloride; Furazolium Tartrate; Fusidate Sodium; Fusidic Acid; Gentamicin Sulfate; Gloximonam; Gramicidin; Haloprogin; Hetacillin; Hetacillin Potassium; Hexedine; Ibafloxacin; Imipenem; Isoconazole; Isepamicin; Isoniazid; Josamycin; Kanamycin Sulfate; Kitasamycin; Levofuraltadone; Levopropylcillin Potassium; Lexithromycin; Lincomycin; Lincomycin Hydrochloride; Lomefloxacin; Lomefloxacin Hydrochloride; Lomefloxacin Mesylate; Loracarbef; Mafenide; Meclocycline; Meclocycline Sulfosalicylate; Megalomicin Potassium Phosphate; Mequidox; Meropenem; Methacycline; Methacycline Hydrochloride; Methenamine; Methenamine Hippurate; Methenamine Mandelate; Methicillin Sodium; Metioprim; Metronidazole Hydrochloride; Metronidazole Phosphate; Mezlocillin; Mezlocillin Sodium; Minocycline; Minocycline Hydrochloride; Mirincamycin Hydrochloride; Monensin; Monensin Sodium; Nafcillin Sodium; Nalidixate Sodium; Nalidixic Acid; Natamycin; Nebramycin; Neomycin Palmitate; Neomycin Sulfate; Neomycin Undecylenate; Netilmicin Sulfate; Neutramycin; Nifuradene; Nifuraldezone; Nifuratel; Nifuratrone; Nifurdazil; Nifurimide; Nifurpirinol; Nifurquinazol; Nifurthiazole; Nitrocycline; Nitrofurantoin; Nitromide; Norfloxacin; Novobiocin Sodium; Ofloxacin; Ormetoprim; Oxacillin Sodium; Oximonam; Oximonam Sodium; Oxolinic Acid; Oxytetracycline; Oxytetracycline Calcium; Oxytetracycline Hydrochloride; Paldimycin; Parachlorophenol; Paulomycin; Pefloxacin; Pefloxacin Mesylate; Penamecillin; Penicillin G

Benzathine; Penicillin G Potassium; Penicillin G Procaine; Penicillin G Sodium; Penicillin V; Penicillin V Benzathine; Penicillin V Hydrabamine; Penicillin V Potassium; Pentizidone Sodium; Phenyl Aminosalicylate; Piperacillin Sodium; Pirbenicillin Sodium; Piridicillin Sodium; Pirlimycin Hydrochloride; Pivampicillin Hydrochloride; Pivampicillin Pamoate; Pivampicillin Probenate; Polymyxin B Sulfate; Porfiromycin; Propikacin; Pyrazinamide; Pyrithione Zinc; Quindecamine Acetate; Quinupristin; Racephenicol; Ramoplanin; Ranimycin; Relomycin; Repromicin; Rifabutin; Rifametane; Rifamexil; Rifamide; Rifampin; Rifapentine; Rifaximin; Rolitetracycline; Rolitetracycline Nitrate; Rosaramicin; Rosaramicin Butyrate; Rosaramicin Propionate; Rosaramicin Sodium Phosphate; Rosaramicin Stearate; Rosoxacin; Roxarsone; Roxithromycin; Sancycline; Sanfetrinem Sodium; Sarmoxicillin; Sarpicillin; Scopafungin; Sisomicin; Sisomicin Sulfate; Sparfloxacin; Spectinomycin Hydrochloride; Spiramycin; Stallimycin Hydrochloride; Steffimycin; Streptomycin Sulfate; Streptonicozid; Sulfabenz; Sulfabenzamide; Sulfacetamide; Sulfacetamide Sodium; Sulfacytine; Sulfadiazine; Sulfadiazine Sodium; Sulfadoxine; Sulfalene; Sulfamerazine; Sulfameter; Sulfamethazine; Sulfamethizole; Sulfamethoxazole; Sulfamonomethoxine; Sulfamoxole; Sulfanilate Zinc; Sulfanitran; Sulfasalazine; Sulfasomizole; Sulfathiazole; Sulfazamet; Sulfisoxazole; Sulfisoxazole Acetyl; Sulfisoxazole Diolamine; Sulfomyxin; Sulopenem; Sultamicillin; Suncillin Sodium; Talampicillin Hydrochloride; Teicoplanin; Temafloxacin Hydrochloride; Temocillin; Tetracycline; Tetracycline Hydrochloride; Tetracycline Phosphate Complex; Tetroxoprim; Thiamphenicol; Thiphencillin Potassium; Ticarcillin Cresyl Sodium; Ticarcillin Disodium; Ticarcillin Monosodium; Ticlatone; Tiodonium Chloride; Tobramycin; Tobramycin Sulfate; Tosufloxacin; Trimethoprim; Trimethoprim Sulfate; Trisulfapyrimidines; Troleandomycin; Trospectomycin Sulfate; Tyrothricin; Vancomycin; Vancomycin Hydrochloride; Virginiamycin; Zorbamycin.

Anti-mycobacterials include Myambutol (Ethambutol Hydrochloride), Dapsone (4,4'-diaminodiphenylsulfone), Paser Granules (aminosalicylic acid granules), Priflin (rifapentine), Pyrazinamide, Isoniazid, Rifadin (Rifampin), Rifadin IV, Rifamate (Rifampin and Isoniazid), Rifater (Rifampin, Isoniazid, and Pyrazinamide), Streptomycin Sulfate and Trecator-SC (Ethionamide).

Anti-virals include amantadine and rimantadine, ribivarin, acyclovir, vidarabine, trifluorothymidine, ganciclovir, zidovudine, retinovir, and interferons.

Anti-virals further include Acemannan; Acyclovir; Acyclovir Sodium; Adefovir; Alovudine; Alvircept Sudotox; Amantadine Hydrochloride; Aranotin; Arildone; Atevirdine

Mesylate; Avridine; Cidofovir; Cipamfylline; Cytarabine Hydrochloride; Delavirdine Mesylate; Desciclovir; Didanosine; Disoxaril; Edoxudine; Enviradene; Enviroxime; Famciclovir; Famotine Hydrochloride; Fiacitabine; Fialuridine; Fosarilate; Foscamet Sodium; Fosfonet Sodium; Ganciclovir; Ganciclovir Sodium; Idoxuridine; Kethoxal; Lamivudine; Lobucavir; Memotine Hydrochloride; Methisazone; Nevirapine; Penciclovir; Pirodavir; Ribavirin; Rimantadine Hydrochloride; Saquinavir Mesylate; Somantadine Hydrochloride; Sorivudine; Statolon; Stavudine; Tilorone Hydrochloride; Trifluridine; Valacyclovir Hydrochloride; Vidarabine; Vidarabine Phosphate; Vidarabine Sodium Phosphate; Viroxime; Zalcitabine; Zidovudine; Zinviroxime and integrase inhibitors.

Anti-fungals include imidazoles and triazoles, polyene macrolide antibiotics, griseofulvin, amphotericin B, and flucytosine. Antiparasites include heavy metals, antimalarial quinolines, folate antagonists, nitroimidazoles, benzimidazoles, avermectins, praxiquantel, ornithine decarboxylase inhbitors, phenols (e.g., bithionol, niclosamide); synthetic alkaloid (e.g., dehydroemetine); piperazines (e.g., diethylcarbamazine); acetanilide (e.g., diloxanide furonate); halogenated quinolines (e.g., iodoquinol (diiodohydroxyquin)); nitrofurans (e.g., nifurtimox); diamidines (e.g., pentamidine); tetrahydropyrimidine (e.g., pyrantel pamoate); sulfated naphthylamine (e.g., suramin).

Other anti-microbials include Difloxacin Hydrochloride; Lauryl Isoquinolinium Bromide; Moxalactam Disodium; Omidazole; Pentisomicin; Sarafloxacin Hydrochloride; Protease inhibitors of HIV and other retroviruses; Integrase Inhibitors of HIV and other retroviruses; Cefaclor (Ceclor); Acyclovir (Zovirax); Norfloxacin (Noroxin); Cefoxitin (Mefoxin); Cefuroxime axetil (Ceftin); Ciprofloxacin (Cipro); Aminacrine Hydrochloride; Benzethonium Chloride : Bithionolate Sodium; Bromchlorenone; Carbamide Peroxide; Cetalkonium Chloride; Cetylpyridinium Chloride : Chlorhexidine Hydrochloride; Clioquinol; Domiphen Bromide; Fenticlor; Fludazonium Chloride; Fuchsin, Basic; Furazolidone; Gentian Violet; Halquinols; Hexachlorophene : Hydrogen Peroxide; Ichthammol; Imidecyl Iodine; Iodine; Isopropyl Alcohol; Mafenide Acetate; Meralein Sodium; Mercufenol Chloride; Mercury, Ammoniated; Methylbenzethonium Chloride; Nitrofurazone; Nitromersol; Octenidine Hydrochloride; Oxychlorosene; Oxychlorosene Sodium; Parachlorophenol, Camphorated; Potassium Permanganate; Povidone-Iodine; Sepazonium Chloride; Silver Nitrate; Sulfadiazine, Silver; Symclosene; Thimerfonate Sodium; Thimerosal: Troclosene Potassium.

Conditions relating to immune deficiency may also be treated using agents of the invention. These conditions include congenital deficiencies, some of which are described in greater detail herein. Examples include the syndromes commonly referred to as congenital disorder of glycosylation (CDG). Another congenital indication is the immunoglobulin deficiency common variable immunodeficiency (CVID) which is characterized by low IgG and IgA, and in some instances low IgM. Subjects having CVID can present with other clinical manifestations including gastrointestinal problems, granulomatous inflammation, cutaneous features, unusual presentations of enteroviral and mycoplasma infection, an increased incidence of autoimmunity, and a predisposition to lymphoma and stomach cancer.

Other congenital indications include agammaglobulinemias such as Bruton's agammaglobulinemia and congenital hypogammaglobulinemia, selective immunoglobulin A deficiency, and severe combined immunodeficiency (i.e., SCID, a T cell deficiency). Immune deficiencies that include low or no immunoglobulin production can be treated using agents of the invention alone, and in some instances, preferably with the antibodies described herein. Other immune deficiencies include amyotrophic lateral sclerosis (ALS), systemic lupus erythematosus, rheumatoid arthritis, Hashimoto's disease, chronic immune thrombocytopenic purpura (chronic ITP), and the like.

In still other embodiments, the subject may be genetically immunocompromised, meaning that they harbor a genetic mutation that renders them immunocompromised even in the absence of an infection or exogenous procedure. Such subjects may have for example a genetic mutation such as in agammaglobulinemia or SCID. These subjects may be treated according to the invention routinely or only when they are at a higher risk of, for example, developing an infectious disease e.g., when traveling to a region where infections are common, when having surgery, when having a skin abrasion, etc.

Agents of the invention may be therapeutically and prophylactically useful for conditions which are responsive to IFN therapy. The IFN therapy may be IFNα, IFNβ, or IFNγ therapy, but is not so limited. Examples of conditions responsive to IFN therapy include multiple sclerosis, tuberculosis, chronic Epstein Barr Virus (EBV) infection, and chronic hepatitis (e.g., chronic hepatitis C), viral hepatitis (e.g., hepatitis C), hepatocellular carcinoma, Kaposi's Sarcoma (AIDS-related), thick primary melanomas, and regional lymph node metastases. Examples of conditions responsive to IFNγ therapy include but are not limited to viral infections and associated diseases and cancer.

One advantage of using agents of the invention in place of IFN therapy may be that agents are less expensive and easier to administer than IFN. These and other conditions can be immunosuppressive and therefore the agents can be used to enhance immunity in such subjects. Other chronic immunosuppressive conditions can arise from pharmaceutical use such as the deliberate use of antiinflammatories such as cox-1 or cox-2 inhibitors celecoxib (Celebrex), rofecoxib (Vioxx), naproxen (Naprosyn), non-steroidal anti-inflammatory drugs (NSAIDS) such as ibuprofen (Motrin, Advil), fenoprofen, indomethacin, and valdecoxib (Bextra), and aspirin; substance abuse such as the alcoholism, intravenous drug use, morphine use; chronic infections or disease states such as gingivitis, osteomyelitis, diabetes types I and II, chronic granulomas, Pneumocystis carinii pneumonia (PCP) infection, recurrent fungal/yeast infections, non-Hodgkin's lymphoma, and Kaposi's Sarcoma.

The invention seeks to enhance other forms of immunotherapy including dendritic cell vaccines. These vaccines generally include dendritic cells loaded *ex vivo* with antigens such as tumor-associated antigens. The dendritic cells can be incubated with the antigen, thereby allowing for antigen processing and expression on the cell surface, or the cells may simply be combined with the antigen prior to injection *in vivo.* Alternatively, the dendritic cells may be activated *in vitro* and then re-infused into a subject in the activated state. Agents of the invention can be combined with the dendritic cells in all of these embodiments. Examples of dendritic cell based vaccines include autologous rumour antigen-pulsed dendritic cells (advanced gynaecological malignancies); blood-derived dendritic cells loaded *ex vivo* with prostate cancer antigen (Provenge; Dendreon Corporation); blood-derived dendritic cells loaded *ex vivo* with antigen for multiple myeloma and other B-cell malignancies (Mylovenge; Dendreon Corporation); and blood-derived dendritic cells loaded *ex vivo* with antigen for cancers expressing the HER-2/neu proto-oncogene (APC8024; Dendreon Corporation); xenoantigen (e.g., PAP) loaded dendritic cells, and the like.

The agents of the invention may also be used with normal and hyper-immune globulin therapy. Normal immune globulin therapy utilizes an antibody product from the serum of normal blood donors. This pooled product contains low titers of antibody to a wide range of antigens such as those of infectious pathogens (e.g., bacteria, viruses such as hepatitis A, parvovirus, enterovirus, fungi and parasites). Hyper-immune globulin therapy utilizes antibodies which are prepared from the serum of individuals who have high titers of an antibody to a particular antigen. The antibodies may be those that are currently used or in development for treating infectious diseases. Examples include zoster immune globulin

(useful for the prevention of varicella-zoster in immunocompromised children and neonates), human rabies immunoglobulin (useful in the post-exposure prophylaxis of a subject bitten by a rabid animal), hepatitis A or B immune globulin (useful in the prevention of hepatitis A or B virus, especially in a subject exposed to the virus), RSV immune globulin (useful in the treatment of respiratory syncitial virus infections), tetanus immunoglobulin; measles immunoglobulin (useful in the prevention of infection in immunocompromised or adult subjects); rubella immunoglobulin (useful in the prevention of infection in pregnant female subjects).

Other antibodies for infectious diseases include anti-shiga toxin antibodies, anti-staphylococcal antibodies (Virion Systems), and the like.

The invention provides a method for treating or preventing a cardiovascular disorder in a subject who has or is at risk of developing a cardiovascular disorder wherein the condition would benefit from immune stimulation. The method comprises administering to a subject in need thereof an agent of the invention in an effective amount to treat or prevent the cardiovascular disorder.

The cardiovascular disorder may be a myocardial infarction, myocardial ischemia, angina (stable or unstable), stroke, and peripheral artery disease (e.g., peripheral ischemic cardiovascular disease), transient ischemic attack, claudication(s), vascular occlusion(s), heart failure, arrhythmia, cardiomyopathy, myocarditis, or valvular heart disease. The cardiovascular disorder can be any cardiovascular disorder associated with an atherosclerotic disease.

As used herein, a subject "at risk of developing a cardiovascular disorder" is a subject determined to be at risk according to conventional medical practice. (See, e.g., Harrison's Principles of Experimental Medicine, 15th Edition, McGraw-Hill, Inc., New York). Typically, an individual at risk of developing a cardiovascular disorder has one or more risk factors associated with cardiovascular disease. Such risk factors include family history of a cardiovascular disorder, hypertension, pre-hypertension, hyperlipidemia, elevated level(s) of a marker of systemic inflammation, diabetes, smoking, atherosclerosis, age, etc. In addition, atrial fibrillation, or recent stroke and/or myocardial infarction are important risk factors.

The degree of risk of a cardiovascular event depends on the multitude and the severity or the magnitude of the risk factors that the human subject has. Risk charts and prediction algorithms are available for assessing the risk of cardiovascular events in a human subject based on the presence and severity of risk factors. One such example is the Framingham

Heart Study risk prediction score. The human subject is at an elevated risk of having a cardiovascular event if the subject's 10-year calculated Framingham Heart Study risk score is greater than 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or 20%.

Another method for assessing the risk of a cardiovascular event in a human subject is a global risk score that incorporates a measurement of a level of a marker of systemic inflammation, such as CRP, into the Framingham Heart Study risk prediction score. Other methods of assessing the risk of a cardiovascular event in a human subject include coronary calcium scanning, cardiac magnetic resonance imaging, determining vascular intra-plaque lipid accumulation, and magnetic resonance angiography.

Hyperlipidemia is hypercholesterolemia and/or hypertriglyceridemia. Hypercholesterolemic human subjects and hypertriglyceridemic human subjects are associated with increased incidence of cardiovascular events. A hypercholesterolemic human subject is one who fits the current criteria established for a hypercholesterolemic human subject. A hypertriglyceridemic human subject is one who fits the current criteria established for a hypertriglyceridemic subject. A hypercholesterolemic subject has an LDL level of >160 mg/dL, or >130 mg/dL and at least two risk factors selected from the group consisting of: male gender, family history of premature coronary heart disease, cigarette smoking, hypertension, low HDL (<35 mg/dL), diabetes mellitus, hyperinsulinemia, abdominal obesity, high lipoprotein, and personal history of a cardiovascular event. A hypertriglyceridemic human subject has a triglyceride (TG) level of >250 mg/dL.

Hypertension is defined as a systolic blood pressure > 140 mm Hg, and/or a diastolic pressure >90 mm Hg or both. Pre-hypertension is defined as systolic blood pressure between 115 and 140 mm Hg, and/or a diastolic pressure between 80 and 90 mm Hg.

Obesity is a state of excess adipose tissue mass. Although not a direct measure of adiposity, the most widely used method to gauge obesity is the body mass index (BMI), which is equal to weight/height^2 (in kg/m^2) (See, e.g., Harrison's Principles of Experimental Medicine, 15th Edition, McGraw-Hill, Inc., N.Y.- hereinafter "Harrison's"). Based on data of substantial morbidity, a BMI of 30 is most commonly used as a threshold for obesity in both men and women. A BMI between 25 and 30 should be viewed as medically significant and worthy of therapeutic intervention, especially in the presence of risk factors that are influenced by adiposity, such as hypertension and glucose intolerance. Although often viewed as equivalent to increased body weight, this need not be the case. Lean but very

muscular individuals may be overweight by arbitrary standards without having increased adiposity. Other approaches to quantifying obesity include anthropometry (skin-fold thickness), densitometry (underwater weighing), computed tomography (CT) or magnetic resonance imaging (MRI), and electrical impedance.

Diabetes mellitus is established in a human subject with a fasting plasma glucose level of 120 mg/dL or higher.

An elevated level(s) of a marker of systemic inflammation is a level that is above the average for a healthy human subject population (i.e., human subjects who have no signs and symptoms of disease). When the marker of systemic inflammation is CRP, a CRP level of $\geq$ 1 is considered an elevated level.

The method of the invention may further comprise administering to the subject other therapies to treat or prevent cardiovascular disorders. Therapies for treating or preventing cardiovascular disorders include but are not limited to diet and/or exercise and/or therapies with: anti-lipemic agents, anti-inflammatory agents, anti-thrombotic agents, fibrinolytic agents, anti-platelet agents, direct thrombin inhibitors, glycoprotein II b/IIIa receptor inhibitors, agents that bind to cellular adhesion molecules and inhibit the ability of white blood cells to attach to such molecules (e.g. anti-cellular adhesion molecule antibodies), alpha-adrenergic blockers, beta-adrenergic blockers, cyclooxygenase-2 inhibitors, angiotensin system inhibitor, anti-arrhythmics, calcium channel blockers, diuretics, inotropic agents, vasodilators, vasopressors, and/or any combinations thereof.

Anti-lipemic agents are agents that reduce total cholesterol, reduce LDLC, reduce triglycerides, or increase HDLC. Anti-lipemic agents include statins and non-statin anti-lipemic agents, and/or combinations thereof. Statins are a class of medications that have been shown to be effective in lowering human total cholesterol, LDLC and triglyceride levels. Statins act at the step of cholesterol synthesis. By reducing the amount of cholesterol synthesized by the cell, through inhibition of the HMG-CoA reductase gene, statins initiate a cycle of events that culminates in the increase of LDLC uptake by liver cells. As LDLC uptake is increased, total cholesterol and LDLC levels in the blood decrease. Lower blood levels of both factors are associated with lower risk of atherosclerosis and heart disease, and the statins are widely used to reduce atherosclerotic morbidity and mortality.

Examples of statins include, but are not limited to, simvastatin (Zocor) (U.S. Patent No. 4,444,784), lovastatin (Mevacor) (U.S. Patent No. 4,231,938), pravastatin (Pravachol) (U.S. Patent No. 4,346,227), fluvastatin (Lescol) (U.S. Patent No. 4,739,073), atorvastatin

(Lipitor) (U.S. Patent No. 5,273,995), cerivastatin (Baycol), rosuvastatin (Crestor), pitivastatin and numerous others described in U.S. Patent No. 5,622,985, U.S. Patent No. 5,135,935, U.S. Patent No. 5,356,896, U.S. Patent No. 4,920,109, U.S. Patent No. 5,286,895, U.S. Patent No. 5,262,435, U.S. Patent No. 5,260,332, U.S. Patent No. 5,317,031, U.S. Patent No. 5,283,256, U.S. Patent No. 5,256,689, U.S. Patent No. 5,182,298, U.S. Patent No. 5,369,125, U.S. Patent No. 5,302,604, U.S. Patent No. 5,166,171, U.S. Patent No. 5,202,327, U.S. Patent No. 5,276,021, U.S. Patent No. 5,196,440, U.S. Patent No. 5,091,386, U.S. Patent No. 5,091,378, U.S. Patent No. 4,904,646, U.S. Patent No. 5,385,932, U.S. Patent No. 5,250,435, U.S. Patent No. 5,132,312, U.S. Patent No. 5,130,306, U.S. Patent No. 5,1 16,870, U.S. Patent No. 5,1 12,857, U.S. Patent No. 5,102,91 1, U.S. Patent No. 5,098,931, U.S. Patent No. 5,081,136, U.S. Patent No. 5,025,000, U.S. Patent No. 5,021,453, U.S. Patent No. 5,017,716, U.S. Patent No. 5,001,144, U.S. Patent No. 5,001,128, U.S. Patent No. 4,997,837, U.S. Patent No. 4,996,234, U.S. Patent No. 4,994,494, U.S. Patent No. 4,992,429, U.S. Patent No. 4,970,231, U.S. Patent No. 4,968,693, U.S. Patent No. 4,963,538, U.S. Patent No. 4,957,940, U.S. Patent No. 4,950,675, U.S. Patent No. 4,946,864, U.S. Patent No. 4,946,860, U.S. Patent No. 4,940,800, U.S. Patent No. 4,940,727, U.S. Patent No. 4,939,143, U.S. Patent No. 4,929,620, U.S. Patent No. 4,923,861, U.S. Patent No. 4,906,657, U.S. Patent No. 4,906,624 and U.S. Patent No. 4,897,402.

Examples of statins already approved for use in humans include atorvastatin, cerivastatin, fluvastatin, pravastatin, simvastatin and rosuvastatin. The reader is referred to the following references for further information on HMG-CoA reductase inhibitors: Drugs and Therapy Perspectives (May 12, 1997), 9: 1-6; Chong (1997) Pharmacotherapy 17:1 157-1 177; Kellick (1997) Formulary 32: 352; Kathawala (1991) Medicinal Research Reviews, 11: 121-146; Jahng (1995) Drugs of the Future 20: 387-404, and Current Opinion in Lipidology, (1997), 8, 362-368. Another statin drug of note is compound 3a (S-4522) in Watanabe (1997) Bioorganic and Medicinal Chemistry 5: 437-444.

Non-statin anti-lipemic agents include but are not limited to fibric acid derivatives (fibrates), bile acid sequestrants or resins, nicotinic acid agents, cholesterol absorption inhibitors, acyl-coenzyme A: cholesterol acyl transferase (ACAT) inhibitors, cholesteryl ester transfer protein (CETP) inhibitors, LDL receptor antagonists, farnesoid X receptor (FXR) antagonists, sterol regulatory binding protein cleavage activating protein (SCAP) activators, microsomal triglyceride transfer protein (MTP) inhibitors, squalene synthase inhibitors, and peroxisome proliferation activated receptor (PPAR) agonists.

Examples of fibric acid derivatives include but are not limited to gemfibrozil (Lopid), fenofibrate (Tricor), clofibrate (Atromid) and bezafibrate. Examples of bile acid sequestrants or resins include but are not limited to colesevelam (WelChol), cholestyramine (Questran or Prevalite) and colestipol (Colestid), DMD-504, GT-102279, HBS-107 and S-8921. Examples of nicotinic acid agents include but are not limited to niacin and probucol. Examples of cholesterol absorption inhibitors include but are not limited to ezetimibe (Zetia). Examples of ACAT inhibitors include but are not limited to Avasimibe, CI-976 (Parke Davis), CP-1 13818 (Pfizer), PD-138142-15 (Parke Davis), F1394, and numerous others described in U.S. Patent Nos. 6,204,278, 6,165,984, 6,127,403, 6,063,806, 6,040,339, 5,880,147, 5,621,010, 5,597,835, 5,576,335, 5,321,031, 5,238,935, 5,180,717, 5,149,709, and 5,124,337. Examples of CETP inhibitors include but are not limited to Torcetrapib, CP-529414, CETi-I, JTT-705, and numerous others described in U.S. Patent Nos. 6,727,277, 6,723,753, 6,723,752, 6,710,089, 6,699,898, 6,696,472, 6,696,435, 6,683,099, 6,677,382, 6,677,380, 6,677,379, 6,677,375, 6,677,353, 6,677,341, 6,605,624, 6,586,448, 6,521,607, 6,482,862, 6,479,552, 6,476,075, 6,476,057, 6,462,092, 6,458,852, 6,458,851, 6,458,850, 6,458,849, 6,458,803, 6,455,519, 6,451,830, 6,451,823, 6,448,295, 5,512,548.

One example of an FXR antagonist is Guggulsterone. One example of a SCAP activator is GW532 (GlaxoSmithKline). Examples of MTP inhibitors include but are not limited to Implitapide and R-103757. Examples of squalene synthase inhibitors include but are not limited to zaragozic acids. Examples of PPAR agonists include but are not limited to GW-409544, GW-501516, and LY-5 10929.

Anti-inflammatory agents include Alclofenac; Alclometasone Dipropionate; Algestone Acetonide; Alpha Amylase; Amcinafal; Amcinafide; Amfenac Sodium; Amiprilose Hydrochloride; Anakinra; Anirolac; Anitrazafen; Apazone; Balsalazide Disodium; Bendazac; Benoxaprofen; Benzydamine Hydrochloride; Bromelains; Broperamole; Budesonide; Carprofen; Cicloprofen; Cintazone; Cliprofen; Clobetasol Propionate; Clobetasone Butyrate; Clopirac; Cloticasone Propionate; Cormethasone Acetate; Cortodoxone; Deflazacort; Desonide; Desoximetasone; Dexamethasone Dipropionate; Diclofenac Potassium; Diclofenac Sodium; Diflorasone Diacetate; Diflumidone Sodium; Diflunisal; Difluprednate; Diftalone; Dimethyl Sulfoxide; Drocinonide; Endrysone; Enlimomab; Enolicam Sodium; Epirizole; Etodolac; Etofenamate; Felbinac; Fenamole; Fenbufen; Fenclofenac; Fenclorac; Fendosal; Fenpipalone; Fentiazac; Flazalone; Fluazacort; Flufenamic Acid; Flumizole; Flunisolide Acetate; Flunixin; Flunixin Meglumine; Fluocortin Butyl; Fluorometholone Acetate;

Fluquazone; Flurbiprofen ; Fluretofen; Fluticasone Propionate; Furaprofen; Furobufen; Halcinonide; Halobetasol Propionate; Halopredone Acetate; Ibufenac; Ibuprofen; Ibuprofen Aluminum; Ibuprofen Piconol; Ilonidap; Indomethacin; Indomethacin Sodium; Indoprofen; Indoxole; Intrazole; Isoflupredone Acetate; Isoxepac; Isoxicam; Ketoprofen; Lofemizole Hydrochloride; Lomoxicam; Loteprednol Etabonate; Meclofenamate Sodium; Meclofenamic Acid; Meclorisone Dibutyrate; Mefenamic Acid; Mesalamine; Meseclazone; Methylprednisolone Suleptanate; Momiflumate; Nabumetone; Naproxen; Naproxen Sodium; Naproxol; Nimazone; Olsalazine Sodium; Orgotein; Orpanoxin; Oxaprozin; Oxyphenbutazone; Paranyline Hydrochloride; Pentosan Polysulfate Sodium; Phenbutazone Sodium Glycerate; Pirfenidone; Piroxicam; Piroxicam Cinnamate; Piroxicam Olamine; Piφ rofen; Prednazate; Prifelone; Prodolic Acid; Proquazone; Proxazole; Proxazole Citrate; Rimexolone; Romazarit; Salcolex; Salnacedin; Salsalate; Salycilates; Sanguinarium Chloride; Seclazone; Seπnetacin; Sudoxicam; Sulindac; Suprofen; Talmetacin; Talniflumate; Talosalate; Tebufelone; Tenidap; Tenidap Sodium; Tenoxicam; Tesicam; Tesimide; Tetrydamine; Tiopinac; Tixocortol Pivalate; Tolmetin; Tolmetin Sodium; Triclonide; Triflumidate; Zidometacin; Glucocorticoids; Zomepirac Sodium.

Anti-thrombotic agents and/or fibrinolytic agents include Plasminogen (to plasmin via interactions of prekallikrein, kininogens, Factors XII, XIIIa, plasminogen proactivator, and tissue plasminogen activator TPA]) Streptokinase; Urokinase: Anisoylated Plasminogen-Streptokinase Activator Complex; Pro-Urokinase; (Pro-UK); rTPA (alteplase or activase; r denotes recombinant); rPro-UK; Abbokinase; Eminase; Sreptase Anagrelide Hydrochloride; Bivalirudin; Dalteparin Sodium; Danaparoid Sodium; Dazoxiben Hydrochloride; Efegatran Sulfate; Enoxaparin Sodium; Ifetroban; Ifetroban Sodium; Tinzaparin Sodium; retaplase; Trifenagrel; Warfarin; Dextrans.

Anti-platelet agents include Clopridogrel; Sulfinpyrazone; Aspirin; Dipyridamole; Clofibrate; Pyridinol Carbamate; PGE; Glucagon; Antiserotonin drugs; Caffeine; Theophyllin Pentoxifyllin; Ticlopidine; Anagrelide. Direct thrombin inhibitors include hirudin, hirugen, hirulog, agatroban, PPACK, thrombin aptamers. Glycoprotein Ilb/IIIa receptor Inhibitors are both antibodies and non-antibodies, and include but are not limited to ReoPro (abcixamab), lamifiban, tirofiban.

Agents that bind to cellular adhesion molecules and inhibit the ability of white blood cells to attach to such molecules include polypeptide agents. Such polypeptides include polyclonal and monoclonal antibodies, prepared according to conventional methodology.

Such antibodies already are known in the art and include anti-ICAM 1 antibodies as well as other such antibodies.

Beta-adrenergic receptor blocking agents are a class of drugs that antagonize the cardiovascular effects of catecholamines in angina pectoris, hypertension, and cardiac arrhythmias. Beta-adrenergic receptor blockers include, but are not limited to, atenolol, acebutolol, alprenolol, befunolol, betaxolol, bunitrolol, carteolol, celiprolol, hedroxalol, indenolol, labetalol, levobunolol, mepindolol, methypranol, metindol, metoprolol, metrizoranolol, oxprenolol, pindolol, propranolol, practolol, practolol, sotalolnadolol, tiprenolol, tomalolol, timolol, bupranolol, penbutolol, trimepranol, 2-(3-(l,l-dimethylethyl)-amino-2-hydroxypropoxy)-3-pyridenecarbonitrilHCl, l-butylamino-3-(2,5-dichlorophenoxy)-2-propanol, l-isopropylamino-3-(4-(2-cyclopropylmethoxyethyl)phenoxy)-2-propanol, 3-isopropylamino-1-(7-methylindan-4-yloxy)-2-butanol, 2-(3-t-butylamino-2-hydroxy-propylthio)-4-(5-carbamoyl-2-thienyl)thiazol,7-(2-hydroxy-3-t-butylaminpropoxy)phthalide. The above-identified compounds can be used as isomeric mixtures, or in their respective levorotating or dextrorotating form. Examples of alpha-adrenergic blockers include: doxazocin, prazocin, tamsulosin, and tarazosin.

Cyclooxygenase-2 (COX-2) is a recently identified new form of a cyclooxygenase. Cyclooxygenase is an enzyme complex present in most tissues that produces various prostaglandins and thromboxanes from arachidonic acid. Non-steroidal, antiinflammatory drugs exert most of their antiinflammatory, analgesic and antipyretic activity and inhibit hormone-induced uterine contractions and certain types of cancer growth through inhibition of the cyclooxygenase (also known *as prostaglandin G/H synthase* and/or *prostaglandin-endoperoxide synthase).* Initially, only one form of cyclooxygenase was known, the "constitutive enzyme" or cyclooxygenase-1 (COX-I). It and was originally identified in bovine seminal vesicles.

Cyclooxygenase-2 (COX-2) has been cloned, sequenced and characterized initially from chicken, murine and human sources (See, e.g., U.S. Patent 5,543,297, issued August 6, 1996). This enzyme is distinct from the COX-I . COX-2, is rapidly and readily inducible by a number of agents including mitogens, endotoxin, hormones, cytokines and growth factors. As prostaglandins have both physiological and pathological roles, it is believed that the constitutive enzyme, COX-I, is responsible, in large part, for endogenous basal release of prostaglandins and hence is important in their physiological functions such as the maintenance of gastrointestinal integrity and renal blood flow. By contrast, it is believed that

the inducible form, COX-2, is mainly responsible for the pathological effects of prostaglandins where rapid induction of the enzyme would occur in response to such agents as inflammatory agents, hormones, growth factors, and cytokines. Therefore, it is believed that a selective inhibitor of COX-2 has similar antiinflammatory, antipyretic and analgesic properties to a conventional non-steroidal antiinflammatory drug, and in addition inhibits hormone-induced uterine contractions and also has potential anti-cancer effects, but with reduced side effects. In particular, such COX-2 inhibitors are believed to have a reduced potential for gastrointestinal toxicity, a reduced potential for renal side effects, a reduced effect on bleeding times and possibly a decreased potential to induce asthma attacks in aspirin-sensitive asthmatic subjects, and are therefore useful according to the present invention.

A number of selective COX-2 inhibitors are known in the art. These include, but are not limited to, COX-2 inhibitors described in U.S. Patent Nos. 5,474,995; 5,521,213; 5,536,752; 5,550,142; 5,552,422; 5,604,253; 5,604,260; 5,639,780; 5,677,318; 5,691,374; 5,698,584; 5,710,140; 5,733,909; 5,789,413; 5,817,700; 5,849,943; 5,861,419; 5,922,742; 5,925,631. Additional COX-2 inhibitors are also described in U.S. Patent 5,643,933.

A number of the above-identified COX-2 inhibitors are prodrugs of selective COX-2 inhibitors, and exert their action by conversion *in vivo* to the active and selective COX-2 inhibitors. The active and selective COX-2 inhibitors formed from the above-identified COX-2 inhibitor prodrugs are described in detail in WO 95/00501 , published January 5, 1995, WO 95/18799, published July 13, 1995 and U.S. Patent 5,474,995, issued December 12, 1995. Given the teachings of U.S. Patent 5,543,297, a person of ordinary skill in the art would be able to determine whether an agent is a selective COX-2 inhibitor or a precursor of a COX-2 inhibitor, and therefore part of the present invention.

An angiotensin system inhibitor is an agent that interferes with the function, synthesis or catabolism of angiotensin II. These agents include, but are not limited to, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II antagonists, angiotensin II receptor antagonists, agents that activate the catabolism of angiotensin II, and agents that prevent the synthesis of angiotensin I from which angiotensin II is ultimately derived. The renin-angiotensin system is involved in the regulation of hemodynamics and water and electrolyte balance. Factors that lower blood volume, renal perfusion pressure, or the concentration of Na^+ in plasma tend to activate the system, while factors that increase these parameters tend to suppress its function.

Angiotensin I and angiotensin II are synthesized by the enzymatic renin-angiotensin pathway. The synthetic process is initiated when the enzyme renin acts on angiotensinogen, a pseudoglobulin in blood plasma, to produce the decapeptide angiotensin I. Angiotensin I is converted by angiotensin converting enzyme (ACE) to angiotensin II (angiotensin-[l-8] octapeptide). The latter is an active pressor substance which has been implicated as a causative agent in several forms of hypertension in various mammalian species, e.g., humans.

Angiotensin (renin-angiotensin) system inhibitors are compounds that act to interfere with the production of angiotensin II from angiotensinogen or angiotensin I or interfere with the activity of angiotensin II. Such inhibitors are well known to those of ordinary skill in the art and include compounds that act to inhibit the enzymes involved in the ultimate production of angiotensin II, including renin and ACE. They also include compounds that interfere with the activity of angiotensin II, once produced. Examples of classes of such compounds include antibodies (e.g., to renin), amino acids and analogs thereof (including those conjugated to larger molecules), peptides (including peptide analogs of angiotensin and angiotensin I), pro-renin related analogs, etc. Among the most potent and useful renin-angiotensin system inhibitors are renin inhibitors, ACE inhibitors, and angiotensin II antagonists. In a preferred embodiment of the invention, the renin-angiotensin system inhibitors are renin inhibitors, ACE inhibitors, and angiotensin II antagonists.

Angiotensin II antagonists are compounds which interfere with the activity of angiotensin II by binding to angiotensin II receptors and interfering with its activity. Angiotensin II antagonists are well known and include peptide compounds and non-peptide compounds. Most angiotensin II antagonists are slightly modified congeners in which agonist activity is attenuated by replacement of phenylalanine in position 8 with some other amino acid; stability can be enhanced by other replacements that slow degeneration *in vivo*. Examples of angiotensin II antagonists include but are not limited to peptidic compounds (e.g., saralasin, [(San^{1})(VaI^{5})(AIa^{8})] angiotensin -(1-8) octapeptide and related analogs); N-substituted imidazole-2-one (US Patent Number 5,087,634); imidazole acetate derivatives including 2-N-butyl-4-chloro-l-(2-chlorobenzile) imidazole-5-acetic acid (see Long et al., *J. Pharmacol. Exp. Ther.* 247(1), 1-7 (1988)); 4, 5, 6, 7-tetrahydro-lH-imidazo [4, 5-c] pyridine-6-carboxylic acid and analog derivatives (US Patent Number 4,816,463); N2-tetrazole beta-glucuronide analogs (US Patent Number 5,085,992); substituted pyrroles, pyrazoles, and tryazoles (US Patent Number 5,081,127); phenol and heterocyclic derivatives such as 1, 3-imidazoles (US Patent Number 5,073,566); imidazo-fused 7-member ring

heterocycles (US Patent Number 5,064,825); peptides (e.g., US Patent Number 4,772,684); antibodies to angiotensin II (e.g., US Patent Number 4,302,386); and aralkyl imidazole compounds such as biphenyl-methyl substituted imidazoles (e.g., EP Number 253,310, January 20, 1988); ES8891 (N-moϕ holinoacetyl-(-l-naphthyl)-L-alanyl-(4, thiazolyl)-L-alanyl (35, 45)-4-amino-3-hydroxy-5-cyclo-hexapentanoyl-N-hexylamide, Sankyo Company, Ltd., Tokyo, Japan); SKF108566 (E-alpha-2-[2-butyl-l-(carboxy phenyl) methyl] IH-imidazole-5-yl[methylane]-2-thiophenepropanoic acid, Smith Kline Beecham Pharmaceuticals, PA); Losartan (DUP753/MK954, DuPont Merck Pharmaceutical Company); Remikirin (RO42-5892, F. Hoffman LaRoche AG); A_2 agonists (Marion Merrill Dow) and certain non-peptide heterocycles (G.D.Searle and Company).

Angiotensin converting enzyme (ACE), is an enzyme which catalyzes the conversion of angiotensin I to angiotensin II. ACE inhibitors include amino acids and derivatives thereof, peptides, including di and tri peptides and antibodies to ACE which intervene in the renin-angiotensin system by inhibiting.the activity of ACE thereby reducing or eliminating the formation of pressor substance angiotensin II. ACE inhibitors have been used medically to treat hypertension, congestive heart failure, myocardial infarction and renal disease. Classes of compounds known to be useful as ACE inhibitors include acylmercapto and mercaptoalkanoyl prolines such as captopril (US Patent Number 4,105,776) and zofenopril (US Patent Number 4,316,906), carboxyalkyl dipeptides such as enalapril (US Patent Number 4,374,829), lisinopril (US Patent Number 4,374,829), quinapril (US Patent Number 4,344,949), ramipril (US Patent Number 4,587,258), and perindopril (US Patent Number 4,508,729), carboxyalkyl dipeptide mimics such as cilazapril (US Patent Number 4,512,924) and benazapril (US Patent Number 4,410,520), phosphinylalkanoyl prolines such as fosinopril (US Patent Number 4,337,201) and trandolopril.

Renin inhibitors are compounds which interfere with the activity of renin. Renin inhibitors include amino acids and derivatives thereof, peptides and derivatives thereof, and antibodies to renin. Examples of renin inhibitors that are the subject of United States patents are as follows urea derivatives of peptides (US Patent Number 5,1 16,835); amino acids connected by nonpeptide bonds (US Patent Number 5,1 14,937); di and tri peptide derivatives (US Patent Number 5,106,835); amino acids and derivatives thereof (US Patent Numbers 5,104,869 and 5,095,1 19); diol sulfonamides and sulfmyls (US Patent Number 5,098,924); modified peptides (US Patent Number 5,095,006); peptidyl beta-aminoacyl aminodiol carbamates (US Patent Number 5,089,471); pyrolimidazolones (US Patent Number

5,075,451); fluorine and chlorine statine or statone containing peptides (US Patent Number 5,066,643); peptidyl amino diols (US Patent Numbers 5,063,208 and 4,845,079); N-morpholino derivatives (US Patent Number 5,055,466); pepstatin derivatives (US Patent Number 4,980,283); N-heterocyclic alcohols (US Patent Number 4,885,292); monoclonal antibodies to renin (US Patent Number 4,780,401); and a variety of other peptides and analogs thereof (US Patent Numbers 5,071,837, 5,064,965, 5,063,207, 5,036,054, 5,036,053, 5,034,512, and 4,894,437).

Calcium channel blockers are a chemically diverse class of compounds having important therapeutic value in the control of a variety of diseases including several cardiovascular disorders, such as hypertension, angina, and cardiac arrhythmias (Fleckenstein, *Cir. Res.* v. 52, (suppl. 1), p.13-16 (1983); Fleckenstein, *Experimental Facts and Therapeutic Prospects,* John Wiley, New York (1983); McCaIl, D., *Curr Pract Cardiol,* v. 10, p. 1-1 1 (1985)). Calcium channel blockers are a heterogenous group of drugs that prevent or slow the entry of calcium into cells by regulating cellular calcium channels. (Remington, *The Science and Practice of Pharmacy,* Nineteenth Edition, Mack Publishing Company, Eaton, PA, p.963 (1995)). Most of the currently available calcium channel blockers, and useful according to the present invention, belong to one of three major chemical groups of drugs, the dihydropyridines, such as nifedipine, the phenyl alkyl amines, such as verapamil, and the benzothiazepines, such as diltiazem. Other calcium channel blockers useful according to the invention, include, but are not limited to, amrinone, amlodipine, bencyclane, felodipine, fendiline, flunarizine, isradipine, nicardipine, nimodipine, perhexilene, gallopamil, tiapamil and tiapamil analogues (such as 1993RO-1 1-2933), phenytoin, barbiturates, and the peptides dynorphin, omega-conotoxin, and omega-agatoxin, and the like and/or pharmaceutically acceptable salts thereof.

Diuretics include but are not limited to: carbonic anhydrase inhibitors, loop diuretics, potassium-sparing diuretics, thiazides and related diuretics. Vasodilators include but are not limited to coronary vasodilators and peripheral vasodilators. Inotropic agents include but are not limited to glycosides such as digitalis, digoxin, amrinone and milrinone. Anti-arrhythmics include but are not limited to quinidien, procainamide, disopyramide, moricizine, lidocaine, mexiletine, phenytoin, tocainide, encainide, flecainide, propafenone, indecainide, propranolol, acebutolol, esmolol, amiodarone, bretylium, verapamil, and diltiazem.

The invention also provides a method for stimulating hematopoiesis in a subject. The method involves administering to a subject in need of such a treatment a composition in an

effective amount to increase the number of hematopoietic cells or mature blood cells in the subject, wherein the composition comprises an agent of the invention.

In one important aspect of the invention, the subject has an abnormally low level of hematopoietic cells or mature blood cells and the agent is administered in an amount effective to restore levels of a hematopoietic cell-type or mature blood cell-type to a pre-selected normal or protective level. The invention has particularly important applications in the restoration of normal or protective levels of neutrophils, erythrocytes, and platelets.

Hematopoietic cells as used herein refer to granulocytes (e.g., promyelocytes, neutrophils, eosinophils and basophils), erythrocytes, reticulocytes, thrombocytes (e.g. megakaryoblasts, platelet-producing megakaryocytes and platelets), lymphocytes, monocytes, dendritic cells and macrophages. Mature blood cells consist of mature lymphocytes, platelets, erythrocytes, reticulocytes, granulocytes and macrophages. In certain important aspects of the invention, the agents useful according to the invention increase the number of neutrophils, erythrocytes and platelets. In connection with erythrocytes, the agents may be used to treat, inter alia, a subject who is anemic. In connection with neutrophils, the agents may be used to treat, inter alia, a subject who has drug or radiation-induced neutropenia, chronic idiopathic neutropenia or cyclic neutropenia.

An anemic subject is a subject that has a below normal level of erythrocytes. A neutropenic subject is a subject who has a below normal level of neutrophils. One important aspect of the invention is restoring in a subject "normal" or "protective" hematopoietic cell levels. A "normal" level as used herein can be a level in a control population, which preferably includes subjects having similar characteristics as the treated individual, such as age. The "normal" level can also be a range, for example, where a population is used to obtain a baseline range for a particular group into which the subject falls. The population can also be divided into groups, such as into quadrants, with the lowest quadrant being individuals with the lowest levels of hematopoietic cells and the highest quadrant being individuals having the highest levels of hematopoietic cells. Thus, the "normal" value can depend upon a particular population selected. Preferably, the normal levels are those of apparently healthy subjects which have no prior history of hematopoietic cell disorders. Such "normal" levels, then can be established as preselected values, taking into account the category in which an individual falls. Appropriate ranges and categories can be selected with no more than routine experimentation by those of ordinary skill in the art. Either the mean or another preselected number within the range can be established as the normal preselected value. Likewise, the

level in a subject prior to treatment with a hematopoietic cell inhibitor can be used as the predetermined value.

In general, the normal range for neutrophils is about 1800-7250 per µl (mean -3650); for basophils 0-150 per µl (mean -30); for eosinophils 0-700 per µl (mean -150); for macrophages and monocytes 200-950 per µl (mean -430); for lymphocytes 1500-4000 per µl (mean -2500); for erythrocytes 4.2×10^6 - 6.1×10^6 per µl; and for platelets 100×10^3 - 333×10^3 per µl. The foregoing ranges are at the 95% confidence level.

In connection with certain conditions, the medical community has established certain preselected values. For example, mild neutropenia is characterized as having a count of between 1000 and 2000 per µl, moderate neutropenia at between 500 and 1000 per µl and severe neutropenia at below 500 per µl. Likewise, in adults, a lymphocyte count at less than 1500 is considered a medically undesirable condition. In children the value is less than 3000. Other preselected values will be readily known to those of ordinary skill in the art. The agents useful according to the invention can be used to establish or to re-establish such preselected values, including normal levels.

A protective level of hematopoietic cells is the number of cells required to confer clinical benefit to the patient. The required levels can be equal to or less than the "normal levels". Such levels are well known to those of ordinary skill in the art. For example, a protective level of neutrophils is above 1000 per µl, preferably, at least 1500 per µl.

As used herein, a hematopoietic cell inhibitor is an exogenously-applied agent (such as a drug or radiation treatment) which causes a decrease in the subject of hematopoietic cells and/or mature blood cells. The radiation exposure may be non-therapeutic exposure including but not limited to accidental exposure or deliberate (e.g., wartime) exposure.

The agents useful according to the invention can be administered in conjunction with exogenous growth factors and cytokines which are specifically selected to achieve a particular outcome. For example, if it is desired to stimulate a particular hematopoietic cell type, then growth factors and cytokines which stimulate proliferation and differentiation of such cell type are used. Thus, it is known that interleukins-1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13 and 17 are involved in lymphocyte differentiation. Interleukins 3 and 4 are involved in mast cell differentiation. Granulocyte macrophage colony stimulating factor (GM-CSF), interleukin-3 and interleukin-5 are involved in the eosinophil differentiation. GM-CSF, macrophage colony stimulating factor (M-CSF) and IL-3 are involved in macrophage differentiation. GM-CSF, G-CSF and IL-3 are involved in neutrophil differentiation. GM-CSF, IL-3, IL-6, IL-1 1 and

TPO are involved in platelet differentiation. Flt3 Ligand is involved in dendritic cell growth. GM-CSF, IL-3, and erythropoietin are involved in erythrocyte differentiation. Finally, the self-renewal of primitive, pluripotent progenitor cells capable of sustaining hematopoiesis requires SCF, Flt3 Ligand, G-CSF, IL-3, IL-6 and IL-1 1. Various combinations for achieving a desired result will be apparent to those of ordinary skill in the art. Because the agents useful according to the invention stimulate primitive, non-committed hematopoietic progenitor cells, they can be used in connection with any of the foregoing categories of agents to stimulate specifically the proliferation of a particular hematopoietic cell type. The foregoing factors are well known to those of ordinary skill in the art, and most are commercially available.

The invention also lends itself to a variety of *in vitro* uses. Hematopoietic progenitor cells are preserved or expanded, or their colony forming unit potential increased, *in vitro*. One benefit that can be obtained according to the invention is the stimulation of hematopoietic progenitor cells by the agents useful according to the invention. Another benefit that can be obtained is the effect that the agent can have on stromal cells used in *in vitro* culturing of hematopoietic progenitor cells. *In vitro* culturing of hematopoietic cells is often carried out in the presence of stromal cells. Hematopoietic progenitor cells typically will not survive, proliferate or differentiate for very long periods of time *in vitro* without appropriate growth factor support.

Stromal cell layers are used to supply such growth agents to cultured hematopoietic cells, either by culturing the hematopoietic progenitor cells *in vitro* with such stromal cells or by supplying the hematopoietic progenitor cells with stromal cell-conditioned medium. The agents useful according to the present invention can be used to treat such stromal cells to cause the stromal cells to manufacture and release growth factors. The incubation of stromal cells with the agents useful according to the invention and in medium is for a period of time sufficient to allow the stromal cells to secrete factors into the medium. The medium then can be used to supplement the culture of hematopoietic progenitor cells and other hematopoietic cells.

The culture of hematopoietic cells is with media which is conventional for culturing cells. Examples include RPMI, DM, ISCOVES, etc. The conditions for such culturing also are known to those of ordinary skill in the art. The conditions typically refer to a combination of parameters (e.g. temperature, CO2 and O_2 content, nutritive media, etc.). The time sufficient to increase the number of cells is a time that can be easily determined by a person skilled in the art, and can vary depending on the original number of cells seeded and the amount added

of growth factors and agents useful according to the invention.

The colony forming potential of hematopoietic uncommitted progenitor cells can be increased by *in vitro* culturing of hematopoietic cells. The cells can be obtained from any blood product or organ containing cells of hematopoietic origin. Crude or unfractionated blood products can be enriched for cells having hematopoietic progenitor cell characteristics in ways well known to those of ordinary skill in the art, prior to or after culture with the agents useful according to the invention.

A particularly important aspect of the invention is in the use of the agents for treatment of neutropenia. It is believed that the agents of the invention may stimulate the proliferation of uncommitted progenitor cells. The agents may also stimulate stromal cells to make G-CSF, which is the growth factor critical in the differentiation and production of neutrophils per se. Thus, the patient may have the dual benefit of stimulation of progenitor cells and differentiation of those cells into neutrophils using the agents of the invention. Similar effects may be seen with erythrocytes and platelets. Thus, treatment to restore neutrophils, erythrocytes and platelets form an independent and distinct aspect of the invention.

The invention intends to treat subjects that are not immunocompromised in some instances. Subject that are not immunocompromised (i.e., "non-immunocompromised") are those that have blood cell counts in the normal range. Normal ranges of blood counts are known to the medical practitioner and reference can be made to a standard hematology textbook for such counts. In addition, reference can be made to published PCT application PCT/USOO/14505. Non-immunocompromised subjects can include subjects that have not undergone any treatment that would render them immunocompromised. For example, such subjects may have a cancer but they have not undergone any treatment such as chemotherapy or radiation that would render them immunocompromised. Such subjects also would not inherently be immunocompromised as a result of the cancer. In some important embodiments, the subjects are at risk of developing an infection due to an impending surgical procedure, travel to a region where one or more infections are common, or they have experienced a skin abrasion, for example as a result of a trauma.

In some embodiments, the method intends to treat subjects free of symptoms calling for hemopoietic stimulation. Thus, the invention intends, in certain embodiments, to treat subjects at a time when they are free of symptoms requiring hemopoietic stimulating treatment or to treat subjects who have such symptoms with amounts or dosages or

administration schedules that differ from those used to protect or restore normal or protective levels of hemopoietic cells. A subject who has previously experienced a need for hemopoietic stimulation but has since recovered its hemopoietic cells to normal or at least protective levels may still be treated by the methods described herein.

As used herein, the terms hemopoietic and hematopoietic are used interchangeably to mean all blood cells including myeloid and lymphoid cells. Myeloid cells include erythrocytes (i.e., red blood cells), macrophages, monocytes, granulocytes including neutrophils, eosinophils and basophils, mast cells, megakaryoctyes, platelets and dendritic cells, and lymphoid cells include T and B lymphocytes, thymic dendritic cells and natural killer (NK) cells. Hemopoietic stimulation, as used herein, refers to the increase in hemopoietic cell numbers or activity to normal or protective levels.

An example of a symptom calling for hemopoietic stimulation is hemopoietic cell numbers below normal or protective levels. A "normal" level as used herein may be a level in a control population, which preferably includes subjects having similar characteristics as the treated individual, such as age and sex. The "normal" level can also be a range, for example, where a population is used to obtain a baseline range for a particular group into which the subject falls. Thus, the "normal" value can depend upon a particular population selected. Preferably, the normal levels are those of apparently healthy subjects who have no prior history of hematopoietic cell disorders. Such "normal" levels then can be established as preselected values, taking into account the category in which an individual falls. Appropriate ranges and categories can be selected with no more than routine experimentation by those of ordinary skill in the art. Either the mean or another preselected number within the range can be established as the normal preselected value.

Thus the methods of the invention, according to some embodiments, are directed towards subjects who possess normal or protective levels of hemopoietic cells, as described herein. Subjects with normal or protective levels of hemopoietic cells are considered to have normal hemopoietic activity. Likewise, in some embodiments, the invention is directed for use in subjects who are not immunocompromised. As used herein, the terms immunocompromised and immunosuppressed are used interchangeably. An example of an immunocompromised subject is one infected with HIV and experiencing AIDS-related symptoms such as low CD4+ T lymphocyte levels. In still other embodiments, the methods may be used in subjects who are HIV positive and who may be immunocompromised, provided that the agent is administered in an amount, a dosing regimen, and an administration

schedule that have a therapeutic effect on abnormal proliferation, such as in a Kaposi's sarcoma tumor, but are not therapeutically effective in stimulating hemopoiesis in the subject.

Thus in certain embodiments, the subjects are not myeloid or lymphoid suppressed or are not candidates for treatment with an agent which causes such suppression at the time of treatment with the methods of the instant invention. Myeloid suppressing conditions are those which induce a reduction in myeloid cells such as erythrocytes, neutrophils or platelets, to below protective or normal levels. Exemplary myelosuppressed conditions are hemopoietic malignancies, including leukemia and lymphoma and diseases such as chronic idiopathic neutropenia, cyclic neutropenia, anemia and thrombocytopenia. Similarly, lymphoid suppressing conditions are those which induce a reduction in lymphoid cells such as T lymphocytes. Suppression of lymphoid cells or some myeloid cells such as granulocytes is also referred to as immunosuppression since reduction in these cell types makes an individual susceptible to, *inter alia*, infection. Subjects may be exposed to myeloid, lymphoid or general immune suppressing conditions by the use of either immunosuppressant drugs such as cyclosporin or high dose chemotherapeutic compounds which affect dividing hemopoietic cells. Immuno-suppression may also arise as a result of treatment modalities such as total body irradiation or conditioning regimens prior to bone marrow transplantation. Viral infection, particularly as in the case of infection with human immunodeficiency virus (HIV), may also immunosuppress an individual. In some embodiments, subjects are those which have not been exposed and are not anticipated to be exposed to the above-mentioned conditions. In other embodiments, the instant invention aims to treat subjects who may have been myelosuppressed or immunosuppressed (e.g., by exposure to one or more of the above conditions), provided that at the time of treatment using the methods described herein, the subject has protective or normal levels of hemopoietic cells.

In still other embodiments, the invention aims to treat subjects who may exhibit symptoms calling for hemopoietic stimulation, provided that the agents are administered in doses, routes and schedules that would not result in hemopoietic stimulation, as explained below. In certain embodiments, the methods of the invention are not intended for use in the treatment of malignancies in HIV infected (i.e., HTV positive) subjects who have below normal or below protective levels of hemopoietic cells, unless the agents are used under conditions, such as administration routes, doses or dosing schedules, that are therapeutically effective in treating abnormal cell proliferation, as described herein, and not effective in stimulating hemopoiesis. For example, in some embodiments, the agent may be administered

once a day, or twice a day, or three or more times a day, for more than 7 days, more than 10 days, more than 14 days or more than 20 days in order to achieve, for example, sustained desired systemic levels. In other embodiments, the agent may be given at timed intervals, such as, for example, every two days, every three days, every four days, every week or every two weeks. In still further embodiments, the agent may be delivered intravenously and continuously, for example, or by injection, such as, in single bolus administrations.

The agents of the invention and the other therapeutic agent(s) may be administered simultaneously or sequentially. When the other therapeutic agents are administered simultaneously they can be administered in the same or separate formulations, but are at least administered at the same time (e.g., within minutes of each other). The administration of the other therapeutic agents (such as adjuvants) and the agent of the invention can also be temporally separated, meaning that the therapeutic agents are administered at a different time, either before and/or after, the administration of the agents of the invention. The separation in time between the administration of these agents may be a matter of minutes or it may be longer. Agents of the invention, adjuvants and yet other therapeutic agents can be used together as well.

In some embodiments, the agents of the invention are administered daily for more than 7 days, more than 10 days, more than 14 days or more than 20 days. In still other embodiments, the agent is administered over a period of weeks, or months. In still other embodiments, the agent is delivered on alternate days. For example, the agent is delivered every two days, every three days, every four days, every five days, every six days, every week, or every month.

According to the methods of the invention, the agents of the invention may be administered prior to, concurrent with, or following other treatment (e.g., anti-cancer compounds, surgery or radiation). The administration schedule may involve administering the different agents in an alternating fashion. In other embodiments, the agent may be delivered before and during, or during and after, or before and after treatment with other therapies. In some cases, the agent is administered more than 24 hours before the administration of other treatment. In other embodiments, more than one therapy may be administered to a subject. For example, the subject may receive the agents of the invention, in combination with both surgery and at least one other anti-cancer compound. As another example, the agent may be administered in combination with more than one anti-cancer drug.

In methods particularly directed at subjects at risk of developing a disorder, timing of the administration of the agent of the invention and the other therapeutic agent may be particularly important. For instance, in a subject with a genetic predisposition to cancer, the agents may be administered to the subject on a routine schedule.

A "routine schedule" as used herein, refers to a predetermined designated period of time. The routine schedule may encompass periods of time which are identical or which differ in length, as long as the schedule is predetermined. For instance, the routine schedule may involve administration on a daily basis, every two days, every three days, every four days, every five days, every six days, a weekly basis, a monthly basis or any set number of days or weeks there-between, every two months, three months, four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, twelve months, etc. Alternatively, the predetermined routine schedule may involve administration on a daily basis for the first week, followed by a monthly basis for several months, and then every three months after that. Any particular combination would be covered by the routine schedule as long as it is determined ahead of time that the appropriate schedule involves administration on a certain day.

In some important embodiments, the timing of administration of the agents of the invention is important. Thus, the invention embraces the administration of an agent of the invention, prior to treatment with other conventional therapy. For example, if the subject has cancer, then conventional therapy includes surgical removal of a tumor, radiation therapy, or chemotherapy. It is preferred in some instances to administer the agent of the invention prior to this therapy, and even more preferred to administer the agent of the invention after this therapy as well. Thus, the method would involve both a prime and a boost dose to antigen (with the agents of the invention).

In embodiments involving the administration of agents of the invention and an antibody such as the anti-HER2 antibody trastuzumab (Herceptin™), the antibody may be administered initially in a dose of 4 mg/kg (dose/unit body weight) as a 90 minute infusion followed by a weekly maintenance dose of 2 mg/kg. In embodiments involving the administration of agents of the invention and an antibody such as the anti-CD20 antibody rituximab (Rituxan™), the antibody may be administered in weekly infusions for 4 or 8 doses (i.e., for 4-8 weeks), each dose being 375 mg/m^2 (dose/unit body surface area). Agents of the invention may be administered, twice daily, for a period immediately prior to the initial antibody dose (e.g., 7 days). Since agents of the invention will expand immune effector cells

(e.g., neutrophils, macrophages, eosinophils and T lymphocytes) and direct them to the microenvironment of the tumor, pretreatment with such compounds will accelerate cytotoxicity mediated by the subsequent administration of antibody. Thus, the agents of the invention can be used solely in a pretreatment regime (i.e., prior to exposure to the antibody), or in a combination of pre- and post-treatment administrations. As a non-limiting example of this latter embodiment, pre-treatment with an agent of the invention may be followed by subsequent courses of defined period (e.g., 7 days) administration that could either be concurrent or spaced by intervals (e.g., 7 day pretreatment, 7 day gap, 7 day treatment etc.). Antibody treatment would be continue weekly as currently recommended by the manufacturer (e.g., Genentech, Inc., IDEC Pharmaceuticals, etc.).

The antibody or antibody fragment may be administered together with an agent of the invention in a multi-day cycle. The multi-day cycle be a 2, 3, 4, 5, 6, 7, 8, 9, 10, or more day cycle. The antibody or fragment thereof may be administered on the first day of such a cycle, followed by administration of the agent of the invention for a number of days, which may or may not be consecutive. For example, the agent of the invention may be administered on all remaining days of a multi-day cycle. The agent of the invention may be administered once, twice, thrice, or more times per day as well. The multi-day cycle may be repeated once, twice, thrice, or more times. Alternatively, it may be repeated for a length of time such as a week, a month, two months, or more, depending upon the status of the subject and the therapeutic response observed. As an non-limiting example, the antibody or fragment thereof is administered on the first day of a seven day cycle, and the agent of the invention is administered twice a day for the remaining six days of the seven day cycle. The seven day cycle is performed four times resulting in a 28 day treatment.

If the agent of the invention is administered together with a chemotherapeutic, then it may be desirable in some embodiments to administer the agent of the invention more frequently than the other therapeutic agent. For example, the agent of the invention may be administered daily or twice daily and the other therapeutic agent may be administered once every 2, 3, 4, 5, 6 or 7 days, or every 1, 2, 3 or 4 weeks. The agent of the invention may also be administered less frequently than daily.

A subject shall mean a human or animal including but not limited to a dog, cat, horse, cow, pig, sheep, goat, chicken, rodent e.g., rats and mice, primate, e.g., monkey, and fish or aquaculture species such as fin fish (e.g., salmon) and shellfish (e.g., shrimp and scallops). Subjects suitable for therapeutic or prophylactic methods include vertebrate and invertebrate

species. Subjects can be house pets (e.g., dogs, cats, fish, etc.), agricultural stock animals (e.g., cows, horses, pigs, chickens, etc.), laboratory animals (e.g., mice, rats, rabbits, etc.), zoo animals (e.g., lions, giraffes, etc.), but are not so limited. Although many of the embodiments described herein relate to human disorders, the invention is also useful for treating other nonhuman vertebrates.

In some embodiments, the compositions and methods are intended for use in elderly subjects. As used herein, an elderly subject is one that is at least 50 years old, preferably at least 60 years old, more preferably at least 70 years old, and most preferably at least 75 years old.

The agents of the invention are administered in effective amounts. An effective amount is a dosage of the agent sufficient to provide a medically desirable result. The effective amount will vary with the particular condition being treated, the age and physical condition of the subject being treated, the severity of the condition, the duration of the treatment, the nature of the concurrent or combination therapy (if any), the specific route of administration (e.g., as in the present invention, oral administration or administration by injection) and like factors within the knowledge and expertise of the health practitioner. The dosage may be adjusted by the individual physician or veterinarian, particularly in the event of any complication. It is preferred generally that a maximum dose be used, that is, the highest safe dose according to sound medical judgment. In general, a therapeutically effective amount means that amount necessary to delay the onset of, inhibit the progression of, or halt altogether the particular condition being treated.

Treatment after a disorder has started aims to reduce, ameliorate or altogether eliminate the disorder, and/or its associated symptoms, or prevent it from becoming worse. Treatment of subjects before a disorder has started (i.e., prophylactic treatment) aims to reduce the risk of developing the disorder. As used herein, the term "prevent" refers to the prophylactic treatment of patients who are at risk of developing a disorder (resulting in a decrease in the probability that the subject will develop the disorder), and to the inhibition of further development of an already established disorder.

For example, in connection with methods directed towards treating subjects having a condition characterized by abnormal mammalian cell proliferation, an effective amount to inhibit proliferation would be an amount sufficient to reduce or halt altogether the abnormal mammalian cell proliferation so as to slow or halt the development of or the progression of a cell mass such as, for example, a tumor. As used in the embodiments, "inhibit" embraces all

of the foregoing. In other embodiments, an effective amount will be an amount necessary to extend the dormancy of micrometastases or to stabilize any residual primary tumor cells following surgical or drug therapy.

In connection with infectious diseases, an effective amount is an amount that would treat or prevent infections, that is, they may be used prophylactically in subjects at risk of developing an infection. Thus, an effective amount is that amount which can lower the risk of, slow or perhaps prevent altogether the development of an infection.

In connection with cardiovascular disorders, an effective amount is that amount which can lower the risk of, slow or perhaps prevent altogether the development of a cardiovascular event.

In still other embodiments, the agent is delivered in an amount, a dose, and a schedule which is therapeutically effective in inhibiting proliferation yet which is not therapeutically effective in stimulating hemopoiesis in the subject. In administering the agents of the invention to subjects, dosing amounts, dosing schedules, routes of administration and the like can be selected so as to affect the other known activities of these compounds. For example, amounts, dosing schedules and routes of administration can be selected as described below, whereby therapeutically effective levels for inhibiting proliferation are provided, yet therapeutically effective levels for restoring hemopoietic deficiency are not provided. As another example, local administration to tumors or protected body areas such as the brain may result in therapeutically effective levels for inhibiting proliferation, but may be non-therapeutically effective levels for hemopoietic cell stimulation.

In some embodiments, agents of the invention can be selected that are effective as antiproliferative agents but are relatively ineffective as hemopoietic cell stimulatory or activating agents. Thus, certain subjects who require both hemopoietic stimulation and/or activation and proliferation inhibition may be treated with different agents of the invention simultaneously, one each for the desired therapeutic effect, or with a single agent but in different dosages, schedules, and/or route to achieve both hemopoietic stimulation and proliferation inhibition at therapeutic levels.

Generally, an effective amount typically varies from 0.001 mg/kg to about 1000 mg/kg, preferably from about 0.002 mg/kg to about 200 mg/kg, and most preferably from about 0.003 mg/kg to about 20 mg/kg, in one or more dose administrations daily, for one or more days.

In particularly important embodiments, the agent is administered in amounts of less than or equal to 1.0 mg/kg per day. This includes amounts equal to or less than 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 mg/kg per day. The agents may also be administered in amounts of less than or equal to 0.1 mg/kg per day (e.g., less than or equal to 0.09, 0.08, 0.07, 0.06, 0.5, 0.04, 0.03, 0.02 or 0.01 mg/kg/day). In some important embodiments, the agent of the invention is administered in a dose of about 0.005 mg/kg to less than or equal to 1.0 mg/kg per day, or a dose of about 0.005 mg/kg to less than or equal to 0.1 mg/kg per day.

In certain embodiments, the agents are administered in amounts of 100 μg, 200 μg, 300 μg, 400 μg, or 500 μg per dose, and such doses may be administered once, twice, or more daily. Thus, for example, an effective amount may be two daily doses of 200 μg or 300 μg each of the agent of the invention.

In some embodiments, the administration of the agents of the invention and another therapeutic agent may be additive. In other instances it is synergistic.

Thus, in one aspect of the invention, the agents of the invention and the other therapeutic agent(s) are administered as a synergistic combination in an effective amount to treat or reduce the risk of developing a cancer. As used herein, the term "synergistic" describes an effect resulting from the combination of at least two agents which is greater than the effect of each of the individual agents when used alone. When used together either or both agents may be used at lower doses than would be used if either agent was administered alone. In these embodiments, either agent or both may be administered in a "sub-therapeutic" dose for each alone, the combination, however, being therapeutic.

A "sub-therapeutic dose" as used herein refers to a dosage which is less than that dosage which would produce a therapeutic result in the subject if administered in the absence of the other agent. For example, the sub-therapeutic dose of an anti-cancer antibody is one which would not produce the desired therapeutic result in the subject in the absence of the administration of the agent of the invention. Therapeutic doses of anti-cancer antibodies are well known in the field of medicine for the treatment of cancer. These dosages have been extensively described in references such as Remington's Pharmaceutical Sciences, 18th ed., 1990, or the Physician Desktop Reference; as well as many other medical references relied upon by the medical profession as guidance for the treatment of cancer.

For any compound described herein an effective amount can be initially determined from cell culture assays. In particular, the effective amount of an agent of the invention can be determined using *in vitro* stimulation assays. The stimulation index of immune cells can

be used to determine an effective amount of the particular compound for the particular subject, and the dosage can be adjusted upwards or downwards to achieve the desired levels in the subject.

Effective amounts can also be determined in animal studies. For instance, the effective amount of an agent of the invention and an anti-cancer antibody to induce a synergistic response can be assessed using *in vivo* assays of tumor regression and/or prevention of tumor formation. Relevant animal models include assays in which malignant cells are injected into the animal subjects, usually in a defined site. Generally, a range agent doses are administered into the animal along with a range of anti-cancer antibody doses. Inhibition of the growth of a tumor following the injection of the malignant cells is indicative of the ability to reduce the risk of developing a cancer. Inhibition of further growth (or reduction in size) of a pre-existing tumor is indicative of the ability to treat the cancer. Mice which have been modified to have human immune system elements can be used as recipients of human cancer cell lines to determine the effective amount of the synergistic combination.

The therapeutic agents may be provided in coated capsules, pills, lozenges and the like. The capsule material may be either hard or soft, and as will be appreciated by those skilled in the art, typically comprises a tasteless, easily administered and water soluble compound such as gelatin, starch or a cellulosic material. The capsules are preferably sealed, such as with gelatin bands or the like.. See, for example, Remington: The Science and Practice of Pharmacy, Nineteenth Edition (Easton, Pa.: Mack Publishing Co., 1995), which describes materials and methods for preparing encapsulated pharmaceuticals.

The other therapeutic agents may be administered by any route available as described below. The particular mode selected will depend, of course, upon the agent selected, the condition being treated, the severity of the condition, whether the treatment is therapeutic or prophylactic, and the dosage required for efficacy. The methods of the invention, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces effective levels of the active compounds without causing clinically unacceptable adverse effects.

The administration route of the agents of the invention is not limiting on the administration route of the other therapeutic agents described herein. The agent of the invention may be administered in the same route, and in the same formulation as the other therapeutic agents, or it may be administered in a different route, different formulation, and even on a different schedule.

When using the agent of the invention in subjects in whom the primary site of abnormal proliferation is well delineated and easily accessible, direct administration to the site may be preferred, provided the tumor has not already metastasized. For example, administration by inhalation for lung tumors or by suppositories in the treatment of cervical, ovarian or rectal tumors may be preferred. Likewise, melanoma, for example, may be treated with the agent via topical administration in and around the area of the lesion. hi still other embodiments aimed at the treatment of subjects with breast or prostate cancer, the agents may be delivered by injection directly into the tissue with, for example, a biopsy needle and syringe.

Systemic administration may be preferred in some instances such as, for example, if the subject is known to have or is suspected of having metastases. In this way, all tumor sites, whether primary or secondary may receive the agent. Systemic delivery may be accomplished through, for example, oral administration in an enteric coating, or by injection.

As discussed earlier, the agent may also be delivered to a tumor site during or immediately after a surgical procedure to remove the tumor by lavage into the excision site or by perfusion of the affected tissue with a physiologically acceptable solution containing the agent. Alternatively, the patient may be administered the agent prior to or following the surgical procedure by continuous infusion. In yet other embodiments, a sustained release device, as described below, such as a polymeric implant may be positioned during surgery in the vicinity of the excision site so as to provide a high local concentration of the agent. These latter embodiments may be appropriate to prevent regrowth of the tumor.

The agent of the invention may be administered alone or in combination with the above-described chemotherapies as part of a pharmaceutical composition. Such a pharmaceutical composition may include the agent in combination with any standard physiologically and/or pharmaceutically acceptable carriers which are known in the art. The compositions should be sterile and contain either a therapeutically or prophylactically effective amount of the agent in a unit of weight or volume suitable for administration to a subject. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible solid or liquid filler, diluents or encapsulating substances which are suitable for administration into a subject of the invention. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being co-mingled with the molecules of the present invention, and with each other,

in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy. Pharmaceutically-acceptable further means a non-toxic material that is compatible with a biological system such as a cell, cell culture, tissue, or organism. The characteristics of the carrier will depend on the route of administration. Physiologically and pharmaceutically-acceptable carriers include diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials which are well known in the art.

Suitable buffering agents include: acetic acid and a salt (1-2% w/v); citric acid and a salt (1-3% w/v); boric acid and a salt (0.5-2.5% w/v); and phosphoric acid and a salt (0.8-2% w/v). Suitable preservatives include benzalkonium chloride (0.003-0.03% w/v); chlorobutanol (0.3-0.9% w/v); parabens (0.01-0.25% w/v) and thimerosal (0.004-0.02% w/v).

Compositions suitable for parenteral administration conveniently comprise a sterile aqueous preparation of the agent, which is preferably isotonic with the blood of the recipient. This aqueous preparation may be formulated according to known methods using suitable dispersing or wetting compounds and suspending compounds. The sterile injectable preparation also may be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables. Carrier formulations suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating compounds, and inert gases and the like. The pharmaceutical compositions may conveniently be presented

in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy.

Compositions suitable for oral administration may be presented as discrete units, such as capsules, tablets, lozenges, each containing a predetermined amount of the agent. Other compositions include suspensions in aqueous liquids or non-aqueous liquids such as a syrup, elixir or an emulsion.

For oral administration, the agents can be formulated readily by combining the active compound(s) with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject to be treated. Pharmaceutical preparations for oral use can be obtained as solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Optionally the oral formulations may also be formulated in saline or buffers for neutralizing internal acid conditions or may be administered without any carriers.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the agents may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition,

stabilizers may be added. Microspheres formulated for oral administration may also be used. Such microspheres have been well defined in the art. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration, the agents may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the agents for use according to the present invention may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.,* dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges *of e.g.* gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch. Techniques for preparing aerosol delivery systems are well known to those of skill in the art. Generally, such systems should utilize components which will not significantly impair the biological properties of the therapeutic (see, for example, Sciarra and Cutie, "Aerosols," in Remington's Pharmaceutical Sciences, 18th edition, 1990, pp 1694-1712; incorporated by reference). Those of skill in the art can readily determine the various parameters and conditions for producing aerosols without resort to undue experimentation.

The agents, when it is desirable to deliver them systemically, may be formulated for parenteral administration by injection, *e.g.,* by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.,* in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

The agents may also be formulated in rectal or vaginal compositions such as suppositories or retention enemas, *e.g.,* containing conventional suppository bases such as cocoa butter or other glycerides.

In some embodiments, the antigens or antibodies are administered mucosally. In these and other embodiments, the subject may be passively or actively exposed to an antigen. Passive exposure occurs when the subject comes in contact with an antigen, such as an infectious pathogen, by being in an environment in which the pathogen is present, and unbeknownst to the subject. Active exposure on the other hand occurs when the subject is

deliberately administered an antigen generally for the purpose of vaccination. Passive exposure to infectious pathogens often occurs at the mucosal surfaces such as the oral, nasal, vaginal, penile, and rectal surfaces. Accordingly, the invention embraces exposure of antigens at these surfaces, prior to, substantially simultaneously with, and/or following administration of agents of the invention.

In some embodiments, it is preferred that antigens and antibodies by administered by routes that mimic the routes through which antigens or carcinogens would enter the body of the subject. For example, if the antigen is from a respiratory virus, then in some instances it is preferable to administer the antigen by inhalation. Similarly, if the antigen is from a microbe that is generally transmitted by sexual intercourse, then in some instances it is preferable to administer such antigens or antibodies to a vaginal, penile or rectal surface.

In still other embodiments, the agents are administered locally, and optionally the antigens or antibodies are administered locally as well.

In addition to the formulations described previously, the agents may also be formulated as a depot preparation. Such long acting formulations may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Suitable liquid or solid pharmaceutical preparation forms are, for example, aqueous or saline solutions for inhalation, microencapsulated, encochleated, coated onto microscopic gold particles, contained in liposomes, nebulized, aerosols, pellets for implantation into the skin, or dried onto a sharp object to be scratched into the skin. The pharmaceutical compositions also include granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, drops or preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. For a brief review of methods for drug delivery, see Langer, *Science* 249:1527-1533, 1990, which is incorporated herein by reference.

In yet other embodiments, the preferred vehicle is a biocompatible microparticle or implant that is suitable for implantation into the mammalian recipient. Exemplary bioerodible implants that are useful in accordance with this method are described in PCT International

Application No. PCT/US/03307 (Publication No. WO 95/24929, entitled "Polymeric Gene Delivery System", claiming priority to U.S. patent application serial no. 213,668, filed March 15, 1994). PCT/US/0307 describes a biocompatible, preferably biodegradable polymeric matrix for containing a biological macromolecule. The polymeric matrix may be used to achieve sustained release of the agent in a subject. In accordance with one aspect of the instant invention, the agent described herein may be encapsulated or dispersed within the biocompatible, preferably biodegradable polymeric matrix disclosed in PCT/US/03307. The polymeric matrix preferably is in the form of a microparticle such as a microsphere (wherein the agent is dispersed throughout a solid polymeric matrix) or a microcapsule (wherein the agent is stored in the core of a polymeric shell). Other forms of the polymeric matrix for containing the agent include films, coatings, gels, implants, and stents. The size and composition of the polymeric matrix device is selected to result in favorable release kinetics in the tissue into which the matrix device is implanted. The size of the polymeric matrix devise further is selected according to the method of delivery which is to be used, typically injection into a tissue or administration of a suspension by aerosol into the nasal and/or pulmonary areas. The polymeric matrix composition can be selected to have both favorable degradation rates and also to be formed of a material which is bioadhesive, to further increase the effectiveness of transfer when the devise is administered to a vascular or pulmonary surface. The matrix composition also can be selected not to degrade, but rather, to release by diffusion over an extended period of time.

Both non-biodegradable and biodegradable polymeric matrices may be used to deliver the agents of the invention to the subject. Biodegradable matrices are preferred. Such polymers may be natural or synthetic polymers. Synthetic polymers are preferred. The polymer is selected based on the period of time over which release is desired, generally in the order of a few hours to a year or longer. Typically, release over a period ranging from between a few hours and three to twelve months is most desirable. The polymer optionally is in the form of a hydrogel that can absorb up to about 90% of its weight in water and further, optionally is cross-linked with multivalent ions or other polymers.

In general, the agents of the invention may be delivered using the bioerodible implant by way of diffusion, or more preferably, by degradation of the polymeric matrix. Exemplary synthetic polymers which can be used to form the biodegradable delivery system include: polyamides, polycarbonates, polyalkylenes, polyalkylene glycols, polyalkylene oxides, polyalkylene terepthalates, polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, poly-vinyl

halides, polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes and co-polymers thereof, alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, polymers of acrylic and methacrylic esters, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxy-propyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, cellulose sulphate sodium salt, poly(methyl methacrylate), poly(ethyl methacrylate), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate), polyethylene, polypropylene, poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly(vinyl alcohols), polyvinyl acetate, poly vinyl chloride, polystyrene and polyvinylpyrrolidone.

Examples of non-biodegradable polymers include ethylene vinyl acetate, poly(meth)acrylic acid, polyamides, copolymers and mixtures thereof.

Examples of biodegradable polymers include synthetic polymers such as polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, polyurethanes, poly(butic acid), poly(valeric acid), and poly(lactide-cocaprolactone), and natural polymers such as alginate and other polysaccharides including dextran and cellulose, collagen, chemical derivatives thereof (substitutions, additions of chemical groups, for example, alkyl, alkylene, hydroxylations, oxidations, and other modifications routinely made by those skilled in the art), albumin and other hydrophilic proteins, zein and other prolamines and hydrophobic proteins, copolymers and mixtures thereof. In general, these materials degrade either by enzymatic hydrolysis or exposure to water *in vivo,* by surface or bulk erosion.

Bioadhesive polymers of particular interest include bioerodible hydrogels described by H.S. Sawhney, CP. Pathak and J.A. Hubell in Macromolecules, 1993, 26, 581-328, the teachings of which are incorporated herein, polyhyaluronic acids, casein, gelatin, glutin, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl methacrylates), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate). Thus, the invention provides a composition of the above-described agents of the invention for use as a medicament, methods for preparing the medicament and methods for the sustained release of the medicament *in vivo.*

Other delivery systems can include timed release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the agent of the invention, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include the above-described polymeric systems, as well as polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Patent 5,075,109. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono- di- and tri-glycerides; hydrogel release systems; silastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which the agent is contained in a form within a matrix such as those described in U.S. Patent Nos. 4,452,775, 4,675,189 and 5,736,1 52 and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patent Nos. 3,854,480, 5,133,974 and 5,407,686. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

Use of a long-term sustained release implant may be particularly suitable for treatment of chronic conditions, such as a the suspected presence of dormant metastases. Long-term release, are used herein, means that the implant is constructed and arranged to deliver therapeutic levels of the active ingredient for at least 30 days, at least 60 days and more preferably for several months. Long-term sustained release implants are well-known to those of ordinary skill in the art and include some of the release systems described above.

According to another aspect of the invention, a kit is provided. The kit is a package which houses a container which contains an agent of the invention and also houses instructions for administering the agent of the invention to a subject having a condition described herein such as, for example, an abnormal mammalian cell proliferation. The kit may optionally also contain one or more other therapeutic agents for use in combination therapies as described herein.

The kits may house an entire medicinal course of treatment for a condition described herein. Patient compliance therefore will be enhanced, and an entire prescription can be contained in a single package. Ordinarily, a pharmacist individually fills a dispenser unit with

a medicament once the pharmacist receives a doctor's prescription. Because the dispenser of the invention includes an entire medicinal course of treatment and can always include a specific number of solid oral dosage forms, the package can be pre-filled with the appropriate number of units of medicament for treatment for a particular medical purpose.

The medicinal dispenser is a package defining a plurality of medicinal storage compartments, each compartment for housing an individual unit of medicament. An entire medicinal course of treatment is housed in a plurality of medicinal storage compartments.

A package defining a plurality of medicinal storage compartments may be any type of disposable pharmaceutical package or card which holds medicaments in individual compartments. Preferably the package is a blister package constructed from a card, which may be made from stiff paper material, a blister sheet and backing sheet. Such cards are well known to those of ordinary skill in the art.

The kits may comprise in one container one therapeutic agent such as, for example, an antibody or antibody fragment, preferably formulated and contained for administration by injection, and in another container the agent of the invention, formulated either for injection or in enterically coated form for oral administration. As another example, the kits may comprise in one container both the agent of the invention and an antigen, or a cocktail of antigens. Alternatively, the agents of the invention and the antigens maybe provided in the same kit but in different containers, and in different formulations for different administration routes. In some embodiments, it is preferred to provide all the active agents in a powdered form such as a lyophilized form that can be reconstituted prior to administration to a subject. All the kits of the invention can optionally contain instructions for storage, reconstitution (if applicable) and administration.

In some particular embodiments, the agents of the invention are provided in pharmaceutical compositions and kits intended for administration by injection (e.g., subcutaneous injection) or *via* an enterically coated form such as a pill, capsule and the like. The kits may comprise the agents of the invention separate from the pharmaceutically acceptable carrier. That is the agents of the invention may be provided in a dry form in a vial or ampoule with a septum, and thereby intended for reconstitution with a diluent, acid solution or pharmaceutically acceptable carrier. These carriers are preferably isotonic solutions. In some instances the agents are reconstituted in an acid solution but then neutralized just prior to administration with a diluent having a higher pH. The diluent may be a neutral or basic solution. It is preferred that the agents be reconstituted and/or neutralized

shortly prior to administration (e.g., within 3, 2 or 1 hour or within 30 minutes of administration). If the agent is provided in an acid solution then it may be maintained in that form indefinitely. Accordingly, indefinite storage of the agent can be accomplished either in a dry form or in an acid solution. The kits may also include a plurality of containers reflecting the number of administrations to be given to a subject. If the kit contains a first and second container, then a plurality of these would be present. These kits would include instructions for reconstitution, storage, and use.

The invention will be more fully understood by reference to the following Examples.

Examples

Example 1

This Example illustrates the inhibition of FAP and DPP-IV *in vitro* by cyclopropyl-alanine-boroPro and cyclopentyl-glycine-boroPro. The dose response curves (FIG. 1) indicate that compound concentrations achieving 50% inhibition (IC_{50}) of dipeptidyl peptidase activity were 20 nM and 70 nM for inhibition of FAP by cyclopropyl-alanine-boroPro and cyclopentyl-glycine-boroPro respectively, and less than 8 nM for inhibition of DPP-IV by cyclopropyl-alanine-boroPro or cyclopentyl-glycine-boroPro.

Materials and Methods

In vitro assay of compounds for inhibition of dipeptidyl peptidase activity of recombinant human FAP and DPP- IV: Assay reaction mixtures consisted of 120 µl 50 mM HEPES/Na buffer pH 7.6, 140 mM NaCl, 15 µl of FAP or DPP-IV enzyme preparations in a 96-well plate. Enzyme was pre-warmed at 32^0C, and then 7.5 µl inhibitor added. The assay was then started by addition to FAP of 7.5 µl of an 8 mM solution of tripeptidepeptide substrate, Ala-Gly-Pro-(7-amino-4-trifiuoromethyl coumarin) (Ala-Gly-Pro-AFC; Enzyme System Products, Dublin, CA) diluted from a 0.1 M stock in dimethyl sulfoxide, or by addition to DPP-IV of 7.5 µl of a 8 mM solution of dipeptide substrate, Ala-Pro-(7-amino-4-trifluoromethyl coumarin) (Ala-Pro-AFC; Enzyme System Products, Dublin, CA) diluted from a 0.4 M stock in dimethyl formamide. In the case of DPP-PV, the inhibitor was incubated with the enzyme for 4 min before addition of substrate. Reaction mixtures were incubated at 32°C, and production of the fluorescent AFC product was measured continuously in a fluorometer (excitation, 400 nm; emission 505 nm). Fluorometric readings were made

with a Molecular Dynamics Spectra Max GeminiXS capable of reading 96-well microtiter plates.

Example 2

This Example illustrates the inhibition of DPP-IV, DPP 8, FAP and DPP 2 *in vitro* by Norleucine-boroPro. The dose response curves (FIG. 2) indicate that compound concentrations achieving 50% inhibition (IC_{50}) of dipeptidyl peptidase activity were: 0.6nM for inhibition of DPP-IV, 1.8 to 2.2 nM for inhibition of DPP 8, 23.0 to 30.0 nM for inhibition of FAP and 0.8 to 1.0 nM for inhibition of DPP 2.

Materials and Methods

In vitro assay of compounds for inhibition of dipeptidyl peptidase activity of recombinant human DPP-IV, DPP 8, FAP and DPP 2: Assay reaction mixtures consisted of 120 µl 140 mM NaCl buffered with 50 mM HEPES/Na pH 7.6 for DPP-IV, DPP 8 and FAP, or 140 mM NaCl buffered with 120 µl 100 mM MES/Na pH 5.5 for DPP 2 mixed with 15 µl of each enzyme preparation in a 96-well plate. 15 µl of varying amounts of Norleucine-boroPro or buffer were added so as to obtain a concentration range of 0 to 100,000 nM in the final reaction mixture. After 5-minutes preincubation at room temperature, 15 µl of 1, 2, 4, or 10 mM Ala-ProAFC was added and incubation continued for a further 15 min. Reactions were stopped by addition of 50 µl 1 M sodium acetate and Fluorometric readings (excitation, 400 πτi; emission 505 ran) were made with a Molecular Dynamics Spectra Max GeminiXS capable of reading 96-well microtiter plates.

Example 3

This Example illustrates that cyclopentyl-glycine-boroPro and cyclopropyl-alanine-boroPro stimulate cytokine and chemokine production by cultured human bone marrow stromal cells *in vitro*, as indicated by measurement of the levels of interleukin-8 (IL-8) and granulocyte colony stimulating factor (G-CSF) in culture supernatants (FIG. 3).

Materials and Methods

Human bone marrow stromal cell cultures: Samples of normal human bone marrow were purchased from Cambrex Bioproducts (Walkersville, MD) and mononuclear cells were purified over Ficoll-Hypaque (Nycomed, Oslo, Norway). Human stromal layers were

established by seeding 4 x 10^7 mononuclear cells into T75 flasks (Coming) containing 20 ml MyeloCult medium (Stem Cell Technologies, Vancouver, BC) supplemented with 10^{-6} M hydrocortisone (Sigma, St. Louis, MO) and incubation at 37^{0}C in 100% humidified 5% CO_2 in air. After one week, half the medium was exchanged, and the cultures incubated for approximately one week more, after which time, a semi-confluent cell layer was formed. Stromal cells were harvested by trypsinization using standard technique and 10^5 cells/well were seeded in multi-well plates in 1 ml of fully supplemented DMEM (InVitrogen, Carlsbad, CA). Cyclopentyl-glycine-boroPro or cyclopropyl-alanine-boroPro were each added to triplicate multiwell cultures at concentrations of 10^{-5}, 10^{-6} or 10^{-7} M. Multiwell cultures without the addition of amino boronic dipeptides served as controls.

Assay ofIL-8 and G-CSF supernatant levels in stromal cell cultures: After incubation of multi-well cultures for 24 hours, supernatant concentrations of human IL-8 and G-CSF were determined by Quantikine enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. ELISA was performed in duplicate for each sample. IL-8 and G-CSF concentrations were compared between cultures containing amino boronic dipeptides and control cultures.

Example 4

This Example illustrates that cyclopentyl-glycine-boroPro stimulates cytokine and chemokine production in cultures of adherent monocytes derived from normal human blood incubated in the presence of the mouse MM46T fibroblast-derived cell line (FIGs. 4A to C) or in the presence of conditioned medium derived from cultures of MM46T cells (FIG. 4D), as indicated by measurement of the levels human IL-1β (FIG. 4A and D), human G-CSF (FIG. 4B), or mouse CXCL1/KC (FIG. 4C) *in vitro.*

Materials and Methods

Human monocyte cell cultures: Previously frozen normal human peripheral blood mononuclear cells (Cambrex Bioproducts, Walkersville, MD) were incubated for one hour in 48-well plates at a concentration of 1.5 x 10^6 cells/ml. The adherent monocytes were washed three times and incubated for 18 hours with either no addition or cyclopentyl-glycine-boroPro at concentrations ranging from 0.1 to 100 nM in 10-fold increments. The 18 hour incubation of the monocyte was performed either in the presence of the mouse fibroblast-derived MM46T cells (ATCC) at a concentration of 5 x 10^4 cells/ml or in the presence of filtered

conditioned medium obtained from cultures of the MM46T cell line as follows. Conditioned medium was collected from MM46T cells incubated for 2 days in RPMI 1640 medium containing 10% fetal bovine serum and fractionated by Amicon Centriplus YM-3 (Millipore, Billerica, MA) filtration to produce a filtrate containing material of ≤ 3 kDa in molecular size. The filtrate was reconstituted with heat-inactivated fetal bovine serum (10%) and used as the culture medium for adherent monocytes incubated with or without cyclopentyl-glycine-boroPro.

Assay of IL-Iβ, G-CSF, and CXCLl/KC supernatant levels in human monocyte cultures: After incubation of multi-well cultures for 18 hours, supernatant concentrations of human IL-I β and G-CSF and mouse CXCL1/KC were determined by Quantikine enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. ELISA was performed in duplicate for samples obtained from cultures performed in triplicate for each concentration of cyclopentyl-glycine-boroPro tested. The cytokine and chemokine concentrations were compared between cultures containing cyclopentyl-glycine-boroPro and control cultures.

Example S

This Example illustrates that cyclopropyl-alanine-boroPro and cyclopentyl-glycine-boroPro can inhibit serum DPP-IV activity and stimulate increased levels of serum KC/CXCL1 in a dose dependent fashion *in vivo* when administered orally (by gavage) to BALB/c mice (FIG. 5). Serum DPP-IV activity was assayed because its inhibition serves to indicate the oral bioavailability of small molecules that inhibit the enzyme. Serum KC/CXCL1 was assayed because it was previously shown to be an indicator of increased levels of cytokines and chemokines in serum of mice administered active amino boronic dipeptide compounds.

Materials and Methods

Assay of serum DPP-IV inhibition and KC/CXCL1 levels in vivo: Varying doses (0.67, 6.7, 67, and 667 nmole/mouse) of cyclopropyl-alanine-boroPro or cyclopentyl-glycine-boroPro dissolved in normal saline were administered to BALB/c mice by gavage. Control mice received the saline vehicle alone. Blood samples were withdrawn from the mice 2 hours after a single administration of each amino boronic dipeptide or vehicle. Serum DPP-IV

activity was assayed in reaction mixtures containing 10 µl serum and 90 µl 140 mM NaCl, 50 mM Hepes/Na, pH 7.6 containing 0.1 ImM of Gly-Pro-AFC. Reactions were incubated for 30 minutes at room temperature and stopped by addition of 100 µl 1 M sodium acetate. Fluorometric readings (excitation, 400 nm; emission, 505 ran) were made with a Molecular Dynamics Spectra Max Gemini XS. Serum concentrations of mouse KC/CXCL1 were determined by Quantikine enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN), according to the manufacturer's instructions. Assays were performed in duplicate for each sample. No detectable levels of KC/CXCL1 were measured in the serum of saline treated mice.

Example 6

This Example illustrates that Norleucine-boroPro can inhibit serum DPP-IV activity and stimulate increased levels of serum KC/CXCL1 in a dose dependent fashion *in vivo* when administered either orally (by gavage) or subcutaneously (by injection) to BALB/c mice (FIG. 6). Serum DPP-IV activity was assayed because its inhibition serves to indicate the oral bioavailability of small molecules that inhibit the enzyme. Serum KC/CXCL1 was assayed because it was previously shown to be an indicator of increased levels of cytokines and chemokines in serum of mice administered active amino boronic dipeptide compounds.

Materials and Methods

Assay of serum DPP-IV inhibition and KC/CXCL1 levels in vivo: Varying doses (0.067, 0.67, 6.7 and 67 nmole/mouse) of Norleucine-boroPro dissolved in normal saline were administered to BALB/c mice by gavage. Control mice received the saline vehicle alone. Blood samples were withdrawn from the mice 2 hours after a single administration of each amino boronic dipeptide or vehicle. Serum DPP-IV activity was assayed in reaction mixtures containing 10 µl serum and 90 µl 140 mM NaCl, 50 mM Hepes/Na, pH 7.6 containing 0.1 ImM of Gly-Pro-AFC. Reactions were incubated for 30 minutes at room temperature and stopped by addition of 100 µl 1 M sodium acetate. Fluorometric readings (excitation, 400 nm; emission, 505 nm) were made with a Molecular Dynamics Spectra Max Gemini XS. Serum concentrations of mouse KC/CXCL1 were determined by Quantikine enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN), according to the manufacturer's instructions. Assays were performed in duplicate for each sample. No detectable levels of KC/CXCL1 were measured in the serum of saline treated mice.

Example 7

This Example illustrates that oral administration of cyclopentyl-glycine-boroPro and cyclohexyl-glycine-boroPro can stimulate antigen-specific T-cell responses to subcutaneously injected synthetic peptides representing MHC class I- and class Il-restricted antigenic epitopes. Compared to untreated naïve mice and to mice injected with peptides with oral administration of saline vehicle alone, significantly increased frequencies of interferon- γ (EFN-γ) producing T-cells were detected by the ELISPOT assay from the spleens of mice administered the peptide antigens and either cyclopentyl-glycine-boroPro or cyclohexyl-glycine-boroPro (FIG. 7). The experiment was performed in BALB/c (H-2^d) mice co-immunized with a MHC class I (L^d) restricted peptide consisting of residues 118-126 of the nucleoprotein from lymphocytic choriomeningitis virus (LCMV NP π$_{8-120}$) and a MHC class II (I-A^d) restricted peptide consisting of residues 323-336 of hen egg ovalbumin (OVA $_{323-336}$)-

Materials and Methods

Immunization of mice and administration ofPT-810 or PT-820: Female BALB/c mice (6-8 week of age) were immunized on days 0 and 7 with equimolar amounts (50 nmol of each per mouse) of LCMV NP 118-126 and OVA 323-336 dissolved in saline and injected subcutaneously at the base of the tail. Cyclopentyl-glycine-boroPro and cyclohexyl-glycine-boroPro were co-administered individually, in separate treatment groups, with peptides on days 0 and 7, and were also given on each flanking day: for example, for priming the mice received PT-810 or PT-820 on days -1, 0, and +1. PT-810 and PT-820 were administered by gavage in 0.2 ml saline at mid-day. On day 0 PT-810/PT-820 and peptides were administered within 60 minutes of each other. Specific T-cell responses from *ex vivo* splenocytes were quantitatively measured 5 weeks after last immunization by IFN-γ ELISPOT.

EliSpot assay of T cell responses in mice: Spleen cells from immunized mice were assayed for their ability to secrete IFN-γ during *in vitro* restimulation with antigenic peptides by an IFN-γ specific ELISPOT assay (mouse IFN-γ ELISPOT; R&D Systems, Minneapolis, MN). Pooled splenocytes from 2 mice per treatment group plated at 5x1 0^5 cells/well were incubated with 1 μg/ml LCMV NP 118-126, 10 μg/ml OVA 323-336, 10 μg/ml Bag 28-39 (class I MHC matched irrelevant control peptide) or no peptide (medium alone). 2.5 μg/ml Con A was incubated with individual splenocyte populations as a positive control to insure

their capacity to secrete IFN-γ. After 18 hr of incubation at 37°, plates were washed and developed according to the kit manufacturer's instructions. Spots were counted using a stereomicroscope and results expressed as IFN-γ spot forming cells (SFC)AO[6] splenocytes. The SFC frequencies shown in Fig. 7 represent the mean and standard deviation of triplicate wells.

Examples using agents having Formula II-V used the L, L version of these agents.

Example 8

This Example describes the production of human DPP 8 and DPP 9 as soluble recombinant proteins. DPP8 cDNA was amplified from cDNA made from total RNA isolated from Caco-2 cell line. Primer sequences are 5' CCAAGCTG GCTAGC TCAAACAGACACCATG gcagcagcaatggaaacagaa 3' (SEQ ID NO: 1) and 5' gtagtca gcggccgc TTA TAT CAC TTT TAG AGC AGC AAT ACG TGA TCC AAG 3' (SEQ ID NO: 2) which contain a Nhel and Notl restriction site respectively. The cDNA was cut with Nhel and Notl and ligated to pSecTag 2B vector (InVitrogen Corp.) cut with the same enzymes. The final clone was pieced together using standard recombinant DNA methods from sections of 3 clones to give no PCR-induced amino acid changes based on DNA sequence in the final purified plasmid (plasmid #197).

DPP9 cDNA was amplified from cDNA made from total RNA isolated from human primary bone marrow stromal cells. Primer sequences are 5' CCAAGCTG TCTAGA TCAAACAGACACCATG gccaccaccgggaccccaacggccga 3' (SEQ ID NO: 3) and 5' gtagtca gcggccgc TCA GAG GTA TTC CTG TAG AAA GTG CAG CAA CGT 3' (SEQ ID NO: 4) which contain a Xbal and Notl restriction site respectively. The cDNA was cut with Nhel and Notl and ligated to pSecTag 2B vector (InVitrogen Corp.) cut with Nhel and Notl.

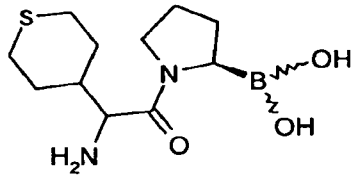
Recombinant DPP8 and DPP9 were prepared identically by transfecting nearly confluent HEK 293T cells with 10-24 micro g DNA and Lipofectamine 2000 reagent (InVitrogen Corp.) in 10 cm dia. dishes according to the manufacturer's instructions. The transfection medium was replaced 15-20 h later with DMEM medium containing 10% fetal calf serum, antibiotics, and glutamine supplement. After 40-48h, the medium was aspirated and plates either frozen at -80C, or lysed immediately to give an extract enriched in the recombinant enzyme. Lysis was done on ice by adding 3.5 ml approx. of 2OmM Tris-Cl pH7.4, 150 mM NaCl, 1% Triton X-100 to the plates. Cells were scraped free of the dish and

pH7.4, 150 mM NaCl, 1% Triton X-100 to the plates. Cells Were scraped free of the dish and transferred to a tube for lysis on ice. After 30 min, debris was pelleted by centrifugation at >8000xg for 5-10 min, and the supernatant used for assays, or stored frozen in aliquots at -8OC.

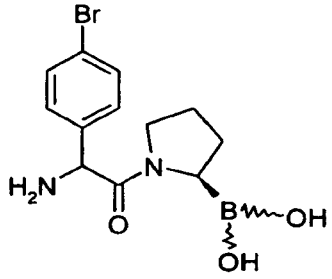
Example 9

This Example illustrates the inhibition of DPP 8 and DPP 9 by the following compounds.

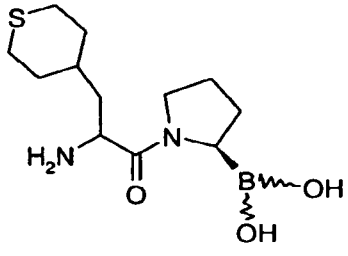
Compound of **Formula VIf,** wherein the side chain is racemic (tetrahydro-thiopyran-4-yl).



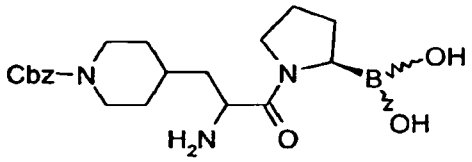
Compound of **Formula VIIf** wherein the amino acid linked to the boroPro is racemic (4-bromophenyl) glycine.



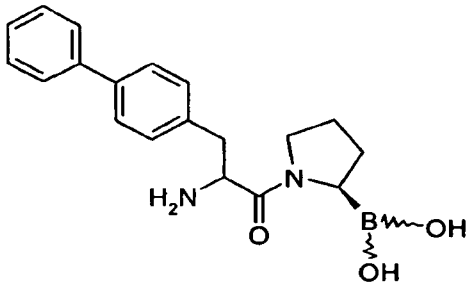
Compound of **Formula VIIIf** wherein the amino acid linked to the boroPro is racemic (4-thiotetrahydropyranyl) alanine.



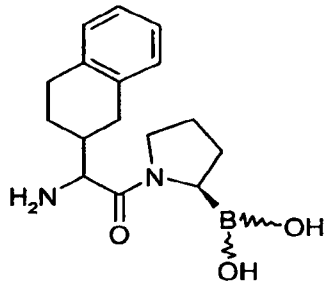
Compound of **Formula** IXf, wherein the amino acid linked to the boroPro is 2-amino-(piperidin-4-yl)-propionic acid and Cbz denotes carboxy benzyl-oxy protecting group.



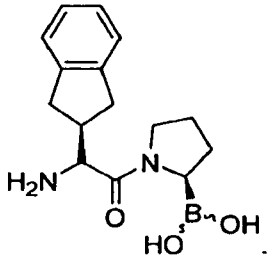
Compound of **Formula Xf** wherein the amino acid linked to the boroPro is 2-amino (3 bi-phenyl-4-yl) propionic acid.



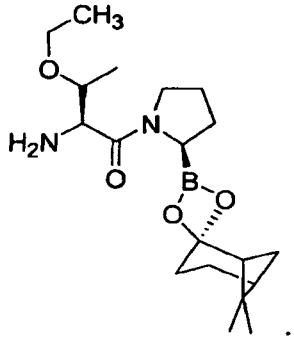
Compound of **Formula XIf,** wherein the N-terminal proximal amino acid is 2-amino-(1,2,3,4-tetra hydro-naphthalen-2-yl) acetic acid and all chiral carbons in are racemic yielding a mixture of 4 diastereomers.



Compound of **Formula XIIe,** wherein the amino acid linked to the boroPro is amino-indan-2-yl acetic acid.



Compound of **Formula XIIIf,** wherein the amino acid linked to the boroPro is (2S,3S)-2-amino-3-ethoxybutanoic acid, and the boronate is protected with the pinanediol group.



The compound concentrations achieving 50% inhibition (IC_{50}) of dipeptidyl peptidase activity of DPP 8 and DPP 9 were found to be in the range of 4 to 80 nM (FIG. 8).

Materials and Methods

Assay reaction mixtures consisted of 90 µl 50 mM potassium phosphate pH7.2, 1 mM EDTA, 0.1% Tween-20, 0.1% bovine serum albumin and 0.1-2 µl enzyme preparations in a 96-well plate. Enzyme was pre-warmed at 32^{0}C, and then 5 µl inhibitor was added. Inhibitor concentrations in the range 0.5 - 10,000 nM were typically used in a 2-fold to 4-fold dilution series. The assay was then started by addition of 5 µl of a 6-8 mM solution of dipeptide substrate, Ala -Pro-(7-amino-4-trifluoromethyl coumarin) (Ala-Pro- AFC; Enzyme System Products, Dublin, CA) diluted from a 0.4 M stock in dimethyl formamide. The inhibitor was incubated with the enzyme for 3-5 min before addition of substrate. Reaction mixtures were incubated at 32°C, and production of the fluorescent AFC product was measured continuously in a fluorometer (excitation, 400 ran; emission 505 run). Fluorometric readings were made with a Molecular Dynamics Spectra Max GeminiXS capable of reading 96-well microtiter plates. Linear rates were fitted to the data and percentage inhibition plotted against inhibitor concentration to determine IC50 values, which were interpolated manually.

***Example** 10*

This Example illustrates the activation of caspase-1 (also called IL-I β converting enzyme or ICE) in cells of the THP-I human monocytic line by the compounds described in Example 9. THP-I cells were incubated with each compound for a total of 11.5 hours and determinations of caspase-1 activity after 2, 3, 4, 5, 6, *1,* 8 and 11.5 hours indicated

progressive activation of caspase-1 (FIG. 9). The compounds activated caspase-1 when added to the THP-I cell cultures at concentrations of 1, 10 and 100 μM.

Materials and Methods

THP-I monocyte cell line (ATCC TIB-202) cells were resuspended at 10^6 cells/ml in serum-free, dye-free growth medium and 50 μl (50,000 cells) of the cell suspension dispensed into a 96-well dark sided assay plate. The Caspase-1 substrate, N-acetyl-WEHD-AFC (WEHD denotes a peptide of sequence Trp-Glu-His-Asp), was diluted in D-PBS (calcium and magnesium free) and 100 μl added to each well so as to obtain a final concentration of 50 μM. Each dipeptidyl peptidase inhibitor prepared as 0.1M solution in 0.IM HCl (with up to 50% v/v DMSO) was diluted in D-PBS and 50 μl was added to the assay plate wells so as obtain a total volume of 200 μl and final concentrations in the range 0.1 - 100 μM. The plates were incubated at 37^0C in a humidified 5% CO_2 atmosphere for a total of 11.5 hours. Fluorometric (excitation, 400 nm; emission, 505 nm) determinations of AFC (7-amino-4-trifluoromethyl coumarin) were made after 2, 3, 4, 5, 6, 7, 8 and 11.5 hours of incubation with a Molecular Dynamics Spectra Max Gemini XS.

Equivalents

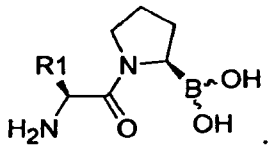
It should be understood that the preceding is merely a detailed description of certain embodiments of the invention. These examples, however, are merely intended to illustrate the embodiments of the invention and are not to be construed to limit the scope of the invention. It therefore should be apparent to those skilled in the art that various modifications and equivalents can be made without departing from the spirit and scope of the invention. It is intended to encompass all such modifications within the scope of the appended claims.

All references, patents and patent publications that are recited in this application are incorporated in their entirety herein by reference.

What is claimed is:

Claims

1. An agent having the structure of **Formula Ie**



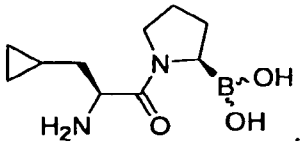
wherein R1 comprises a ring structure or — R3, wherein R3 comprises a ring structure.

2. The agent of claim 1, wherein R1 comprises a five carbon ring structure.

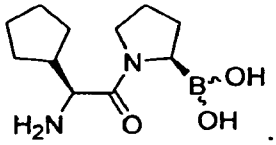
3. The agent of claim 1, wherein R3 comprises a three carbon ring structure.

4. The agent of claim 1, wherein R1 comprises a six carbon ring structure.

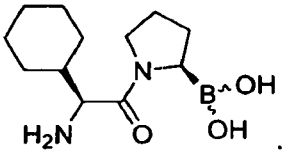
5. The agent of claim 1, wherein the agent has a structure of **Formula He**



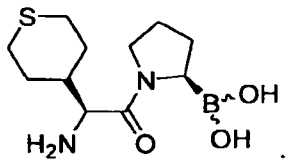
6. The agent of claim 1, wherein the agent has a structure of **Formula HIe**



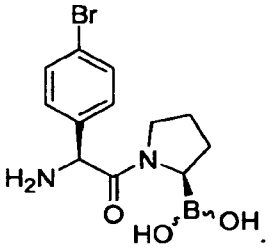
7. The agent of claim 1, wherein the agent has a structure of **Formula IVe**



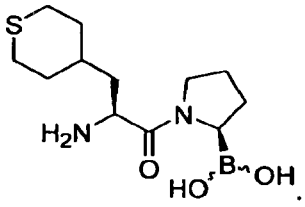
8. The agent of claim 1, wherein the agent has a structure of **Formula VIe**



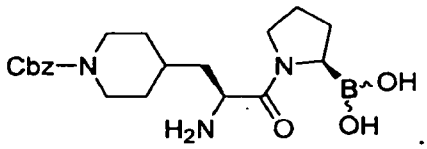
9. The agent of claim 1, wherein the agent has a structure of **Formula VIIe**



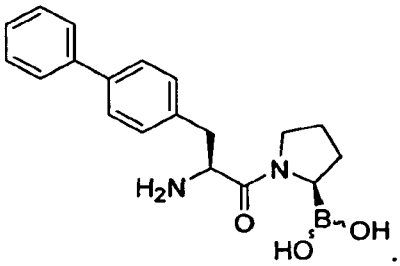
10. The agent of claim 1, wherein the agent has a structure of **Formula VIIIe**



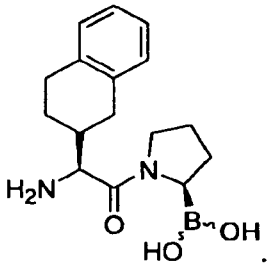
11. The agent of claim 1, wherein the agent has a structure of **Formula IXe**



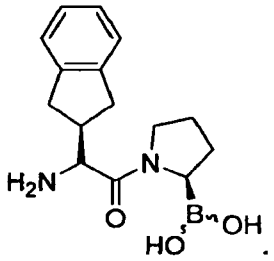
12. The agent of claim 1, wherein the agent has a structure of **Formula** Xe



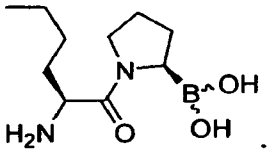
13. The agent of claim 1, wherein the agent has a structure of **Formula XIe**



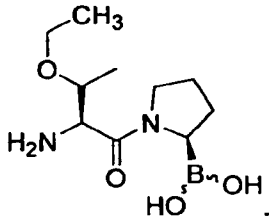
14. The agent of claim 1, wherein the agent has a structure of Formula XIIe



15. An agent having the structure of Formula Ve



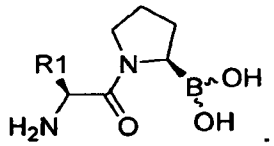
16. An agent having the structure of Formula XIIIe



17. The agent of claim 1-15 or 16, wherein the agent is isolated.

18. A composition comprising

an agent of Formula Ie or a prodrug thereof, wherein Formula Ie has a structure



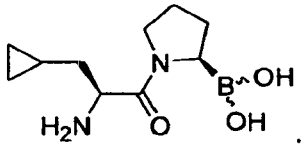
wherein R1 comprises a ring structure or — R3, wherein R3 comprises a ring structure.

19. The composition of claim 18, wherein R1 comprises a five carbon ring structure.

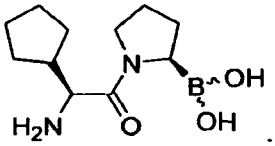
20. The composition of claim 18, wherein R3 comprises a three carbon ring structure.

21. The composition of claim 18, wherein R1 comprises a six carbon ring structure.

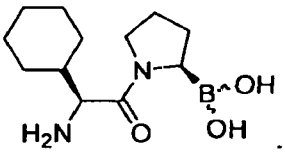
22. The composition of claim 18, wherein the agent has a structure of **Formula lie**



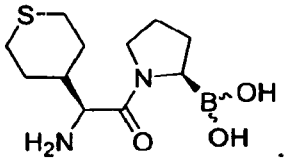
23. The composition of claim 18, wherein the agent has a structure of **Formula IHe**



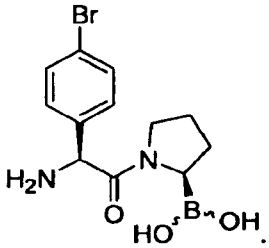
24. The composition of claim 18, wherein the agent has a structure of **Formula IVe**



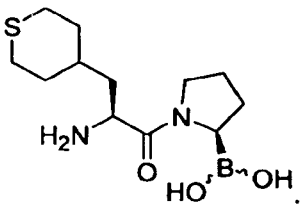
25. The composition of claim 18, wherein the agent has a structure of **Formula VIe**



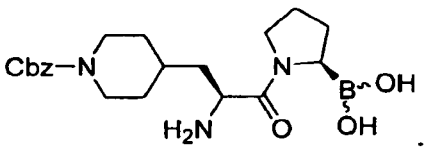
26. The composition of **claim 18,** wherein the agent has a structure of **Formula VlIe**



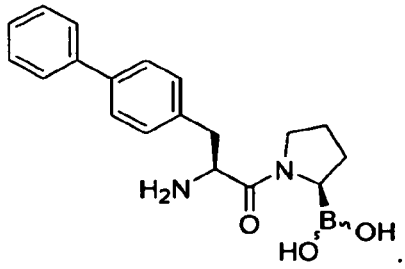
27. The composition of **claim 18,** wherein the agent **has** a structure of **Formula VIIIe**



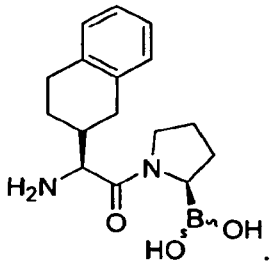
28. The composition of claim **18,** wherein the agent has a structure of **Formula IXe**



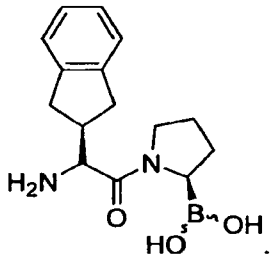
29. The composition of claim 18, wherein the agent has a structure of **Formula Xe**



30. The composition of claim 18, wherein the agent has a structure of **Formula XIe**

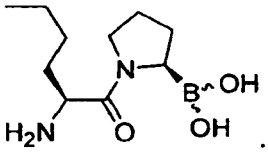


31. The composition of claim 18, wherein the agent has a structure of **Formula XIIe**



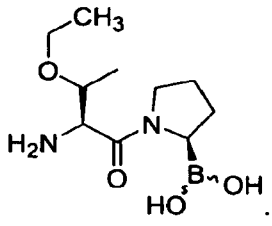
32. **A** composition comprising

an agent of **Formula** Ve or a prodrug thereof, wherein **Formula Ve** has a structure



33. **A** composition comprising

an agent of **Formula XIIIe** or **a** prodrug thereof, wherein Formula **XIIIe** has a structure



34. The composition of claim 18-32 or 33 further comprising a pharmaceutically acceptable carrier.

35. The composition of claim 34, further comprising a second therapeutic agent.

36. The composition of claim 35, wherein the second therapeutic agent is an antigen, an antibody, an antibody fragment, an anti-microbial agent, an anti-cancer agent, a cytokine or an adjuvant.

37. The composition of claim 36, wherein the antigen is a cancer antigen or a microbial antigen.

38. The composition of claim 36, wherein the antibody or antibody fragment is an anti-cancer antibody, an anti-cancer antibody fragment, an anti-microbial antibody, or anti-microbial antibody fragment.

39. The composition of claim 38, wherein the anti-cancer antibody or antibody fragment is trastuzumab (HERCEPTIN), tositumomab (BEXXAR), or alemtuzumab (CAMPATH). mitumomab (BEC2), CeaVac, centuximab (IMC-C225), epratuzumab (LYMPHOCIDE), MDX-2 10, gemtuzumab ozogamicin (MYLOTARG), edrecolomab (PANOREX), pemtumomab (THERAGYN), Zamyl, or ibritumomab tituxetan (ZEVALIN).

40. The composition of claim 38, wherein the anti-cancer antibody or antibody fragment is an anti-HER2/neu antibody, anti-HER2/neu antibody fragment, an anti-CD20 antibody, or anti-CD20 antibody fragment.

4 1. The composition of claim 40, wherein the anti-CD20 antibody is rituxan.

42. The composition of claim 36, wherein the anti-microbial agent is an anti-bacterial agent, an anti-viral agent, an anti-fungal agent, an anti-parasitic agent, or an anti-mycobacterial agent.

43. The composition of claim 18-32 or 33, wherein the agent is present in an effective amount.

44. The composition of claim 18-32 or 33, wherein the composition is formulated for oral delivery.

45. The composition of claim 36, wherein the antibody or antibody fragment is conjugated to a toxin, a chemotherapeutic agent, or a radioisotope.

46. A method for stimulating an immune response in a subject comprising administering to a subject in need thereof the composition of claim A1-A15 or A16 in an amount effective to stimulate an immune response.

47. The method of claim 46, wherein the immune response is an antigen-specific immune response, an innate immune response, or antibody-dependent cell-mediated cytotoxicity.

48. The method of claim 46, wherein the subject has a condition that would benefit from immune stimulation.

49. The method of claim 48, wherein the condition is cancer, infectious disease, cardiovascular disorder, or an IFN-gamma responsive condition.

50. The method of claim 46, wherein the subject is at risk of developing a cancer or an infectious disease.

51. The method of claim 46, wherein the composition is formulated for oral delivery.

52. The method of claim 46, further comprising administering to the subject a second therapeutic agent.

53. The method of claim 52, wherein the second therapeutic agent is an antigen, an antibody, an antibody fragment, an anti-microbial agent, or an anti-cancer agent.

54. The method of claim 53, wherein the antigen is a cancer antigen or a microbial antigen.

55. The method of claim 53, wherein the antibody or antibody fragment is an anti-cancer antibody or anti-cancer antibody fragment or an anti-microbial antibody or anti-microbial antibody fragment.

56. The method of claim 55, wherein the anti-cancer antibody or antibody fragment is an anti-HER2/neu antibody or anti-HER2/neu antibody fragment or an anti-CD20 antibody or anti-CD20 antibody fragment.

57. The method of claim 53, wherein the anti-microbial agent is an anti-bacterial agent, an anti-viral agent, an anti-fungal agent, an anti-parasitic agent or an anti-mycobacterial agent.

58. The method of claim 52, wherein the composition and the second therapeutic agent are administered by different routes.

59. The method of claim 52, wherein the composition and the second therapeutic agent are administered at different times.

60. The method of claim 46, wherein the subject is immunocompromised.

61. The method of claim 60, wherein the immunocompromised subject is genetically immunocompromised.

62. The method of claim 61, wherein the subject has a SCID, agammaglobulinemia, or CDG.

63. The method of claim 61, wherein the subject has an immunoglobulin deficiency that is common variable immunodeficiency.

64. The method of claim 53, wherein the antibody or antibody fragment is conjugated to a toxin, a chemotherapeutic agent or a radioisotope.

65. A method of inhibiting an infectious disease comprising administering to a subject in need thereof the composition of claim Al-Al 5 or A16 in an amount effective to inhibit the infectious disease.

66. The method of claim 65, wherein the subject is at risk of developing an infectious disease.

67. The method of claim 65, wherein the infectious disease is a bacterial infection, a viral infection, a fungal infection, a parasitic infection or a mycobacterial infection.

68. The method of claim 65, wherein the infectious disease a Bacillus anthracis infection (anthrax), Clostridium botulinum infection, Yersinia pestis infection, variola major infection, Francisella tularensis infection, Ebola infection, Marburg infection, Lassa infection, Machupo infection, or Junin infection.

69. The method of claim 67, wherein the bacterial infection is E. coli infection, Staphylococcus infection, Streptococcus infection, Pseudomonas infection, Legionella infection, Pneumococcus infection, Enterobacter infection, Salmonella infection, Listeria infection, and Pasteurella infection.

70. The method of claim 67, wherein the viral infection is HIV infection, Herpes simplex virus infection, cytomegalovirus infection, hepatitis infection, human papilloma virus infection, Epstein Barr virus infection, adenovirus infection, influenza virus infection, respiratory syncytial virus infection, varicella-zoster virus infection, small pox infection, monkey pox infection or SARS infection.

71. The method of claim 67, wherein the fungal infection is candidiasis, ringworm infection, histoplasmosis, blastomycosis, paracoccidioidomycosis, cryptococcosis, aspergillosis, chromomycosis, mycetoma infection, pseudallescheriasis, or tinea versicolor infection.

72. The method of claim 67, wherein the parasitic infection is amebiasis, Trypanosoma cruzi infection, Fascioliasis, Leishmaniasis, Plasmodium infection, Onchocerciasis, Paragonimiasis, Trypanosoma brucei infection, Pneumocystis, Trichomonas vaginalis infection, Taenia infection, Hymenolepsis, Echinococcus infection, Schistosomiasis, neurocysticercosis, Necator americanus infection, or Trichuris trichuria infection.

73. The method of claim 67, wherein the mycobacterial infection is Mycobacterium tuberculosis infection or Mycobacterium leprae infection.

74. The method of claim 65, wherein the composition is formulated for oral delivery.

75. The method of claim 65, further comprising administering to the subject a second therapeutic agent.

76. The method of claim 75, wherein the second therapeutic agent is an antigen, an antibody, an antibody fragment, or an anti-microbial agent.

77. The method of claim 76, wherein the antigen is a microbial antigen.

78. The method of claim 76, wherein the antibody or antibody fragment is an anti¬microbial antibody or antibody fragment.

79. The method of claim 76, wherein the anti-microbial agent is an anti-bacterial agent, an anti-mycobacterial agent, an anti-viral agent, an anti-fungal agent, or an anti-parasitic agent.

80. The method of claim 76, wherein the composition and the second therapeutic agent are administered by different routes.

81. The method of claim 76, wherein the composition and the second therapeutic agent are administered at different times.

82. The method of claim 77, wherein the composition is administered prior to the antigen, antibody, or antibody fragment.

83. The method of claim 76, wherein the composition is administered after the anti¬microbial agent.

84. The method of claim 76, wherein the antibody or antibody fragment is conjugated to a toxin, a chemotherapeutic agent, or a radioisotope.

85. A method of inhibiting a condition characterized by abnormal cell proliferation comprising

administering to a subject in need thereof the composition of claim 18-32 or 33 in an amount effective to inhibit the condition.

86. The method of claim 85, further comprising administering to the subject a second therapeutic agent.

87. The method of claim 86, wherein the second therapeutic agent is an antigen, an antibody, an antibody fragment, an anti-cancer agent, or an enzyme inhibitor.

88. The method of claim 87, wherein the antigen is a cancer antigen.

89. The method of claim 87, wherein the antibody or antibody fragment is an anti-cancer antibody or anti-cancer antibody fragment.

90. The method of claim 89, wherein the anti-cancer antibody or anti-cancer antibody fragment is an anti-HER2/neu antibody, an anti-HER2/neu antibody fragment, an anti-CD20 antibody, or and anti-CD20 antibody fragment.

91. The method of claim 87, wherein the anti-cancer agent is docetaxel (TAXOTERE), cisplatin, gemcitabine, pemetrexed (ALIMTA), erlotinib (TARCEVA), gefitinib (IRESSA), temozolomide (TEMODAR), carboplatin, cyclophosphamide or doxorubicin.

92. The method of claim 87, wherein the enzyme inhibitor is a tyrosine kinase inhibitor, a CDK inhibitor, a MAP kinase inhibitor, or a EGFR inhibitor.

93. The method of claim 86, further comprising then administering the composition of claim 18-32 or 33 alone to the subject.

94. The method of claim 85, wherein the condition is a cancer.

95. The method of claim 94, wherein the cancer is non-small cell lung carcinoma, pancreatic cancer, breast cancer, melanoma, renal cell carcinoma, chronic lymphocytic leukemia, or Non-Hodgkin's lymphoma.

96. The method of claim 94, wherein the cancer is a refractive cancer.

97. The method of claim 85, further comprising administering to the subject an anti-cancer therapy.

98. The method of claim 97, wherein the anti-cancer therapy is radiation or surgery.

99. The method of claim 97, wherein the composition is administered following the anti¬cancer therapy.

100. The method of claim 86, wherein the composition and the second therapeutic agent are administered by different routes.

101. The method of claim 86, wherein the composition and the second therapeutic agent are administered at different times.

102. The method of claim 87, wherein the composition is administered prior to the antigen, antibody, or antibody fragment.

103. The method of claim 86, wherein the composition is administered before and after the second therapeutic agent.

104. The method of claim 87, wherein the antibody or antibody fragment is conjugated to a toxin, a chemotherapeutic agent or a radioisotope.

105. A method for preventing anti-microbial drug resistance in a subject comprising administering to a subject receiving an anti-microbial agent the composition of claim 18-32 or 33 in an amount effective to reduce the risk of resistance to the anti-microbial agent.

106. A method of shortening a vaccination course comprising

administering to a subject in need of immunization the composition of claim 18-32 or 33 in an amount effective to induce an antigen-specific immune response to a vaccine administered in a vaccination course,

wherein the vaccination course is shortened by at least one immunization or at least one day.

107. The method of claim 106, wherein the vaccine is hepatitis vaccine, mumps vaccine, measles vaccine, rubella vaccine, flu vaccine, polio vaccine, tetanus vaccine, DTaP vaccine, HiB vaccine, Pneumococcus vaccine, MMR vaccine, varicella vaccine, DPT vaccine, or Td vaccine.

108. A method for stimulating hematopoiesis in a subject comprising

administering to a subject in need thereof the composition of claim 18-32 or 33 in an effective amount to increase the number of hematopoietic cells or mature blood cells in the subject.

109. The method of claim 108, wherein the subject was exposed to a hematopoietic cell inhibitor.

110. The method of claim 108, wherein the subject will be exposed to a hematopoietic cell inhibitor.

111. The method of claim 108, wherein the subject is neutropenic or anemic.

112. The method of claim 108, further comprising collecting hematopoietic cells from the subject after administration of the composition, and reintroducing the collected cells into the subject.

113. A method for stimulating growth factor production by stromal cells comprising contacting stromal cells with the composition of claim 18-32 or 33 in an amount effective to stimulate growth factor production.

114. The method of claim 113, wherein the contacting occurs *in vitro.*

115. The method of claim 113, wherein the growth factor is G-CSF.

116. The method of claim 113, wherein the growth factor is a chemokine.

117. The method of claim 116, wherein the chemokine is IL-8 or KC/CXCL1 .

118. The method of claim 114, further comprising culturing hematopoietic progenitor cells in the presence of the stromal cells.

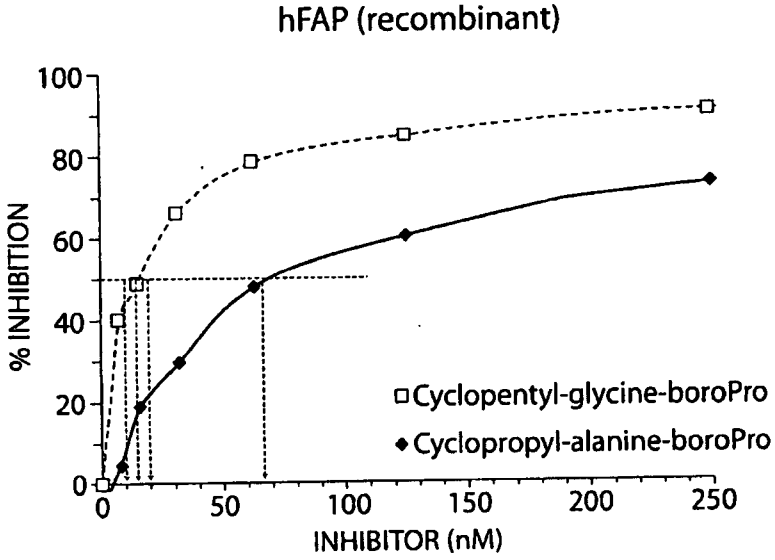

hFAP (recombinant)
100
80
60
40
20
0
% INHIBITION
0
50
100
150
200
250
INHIBITOR (nM)
Cyclopentyl-glycine-boroPro
Cyclopropyl-alanine-boroPro

Fig. 1A

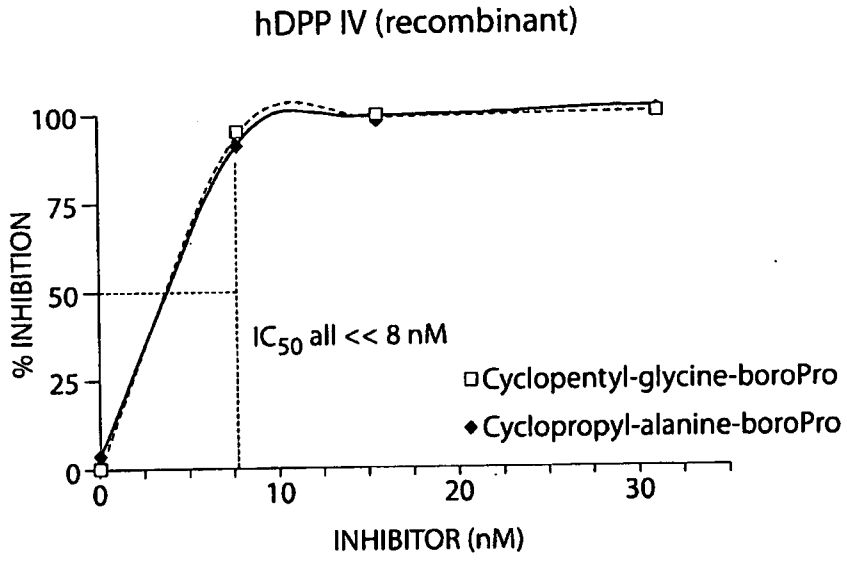

hDPP IV (recombinant)
100
75
50
25
0
% INHIBITION
0
10
20
30
INHIBITOR (nM)
IC50 all << 8 nM
Cyclopentyl-glycine-boroPro
Cyclopropyl-alanine-boroPro

Fig. 1B

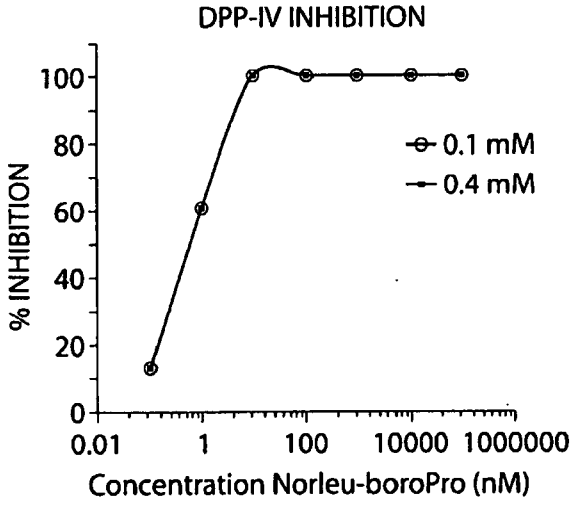

DPP-IV INHIBITION
% INHIBITION
100
80
60
40
20
0
0.01
1
100
10000
1000000
0.1 mM
0.4 mM
Concentration Norleu-boroPro (nM)

Fig. 2A

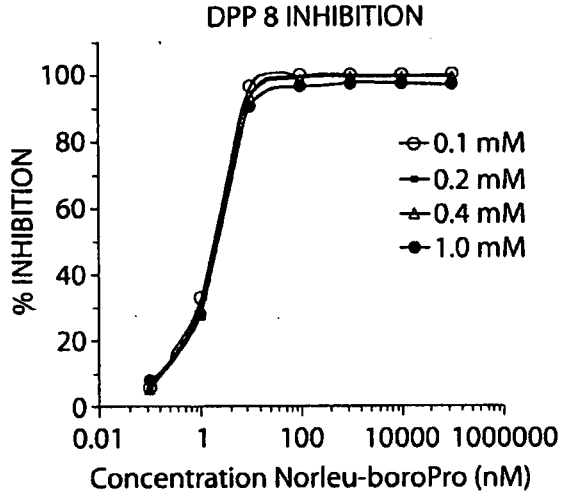

DPP 8 INHIBITION
% INHIBITION
100
80
60
40
20
0
0.01
1
100
10000
1000000
0.1 mM
0.2 mM
0.4 mM
1.0 mM
Concentration Norleu-boroPro (nM)

Fig. 2B

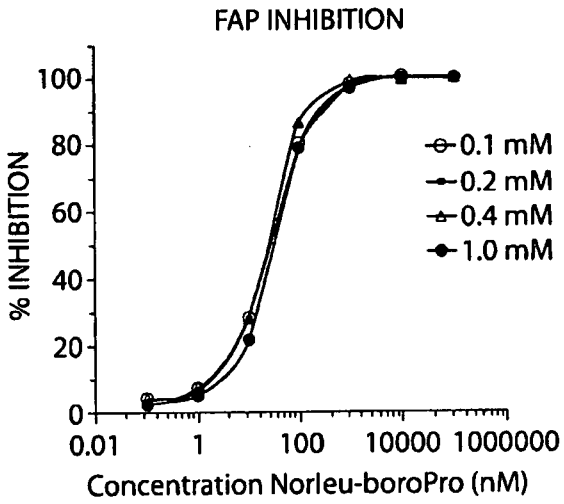

FAP INHIBITION
% INHIBITION
100
80
60
40
20
0
0.01
1
100
10000
1000000
Concentration Norleu-boroPro (nM)
0.1 mM
0.2 mM
0.4 mM
1.0 mM

Fig. 2C

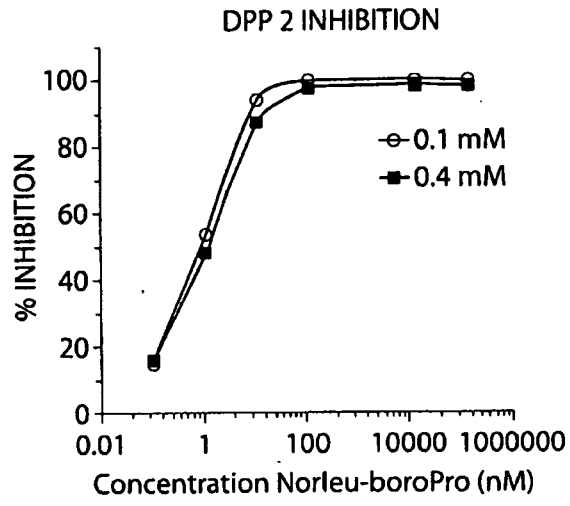

DPP 2 INHIBITION
% INHIBITION
100
80
60
40
20
0
0.01
1
100
10000
1000000
Concentration Norleu-boroPro (nM)
0.1 mM
0.4 mM

Fig. 2D

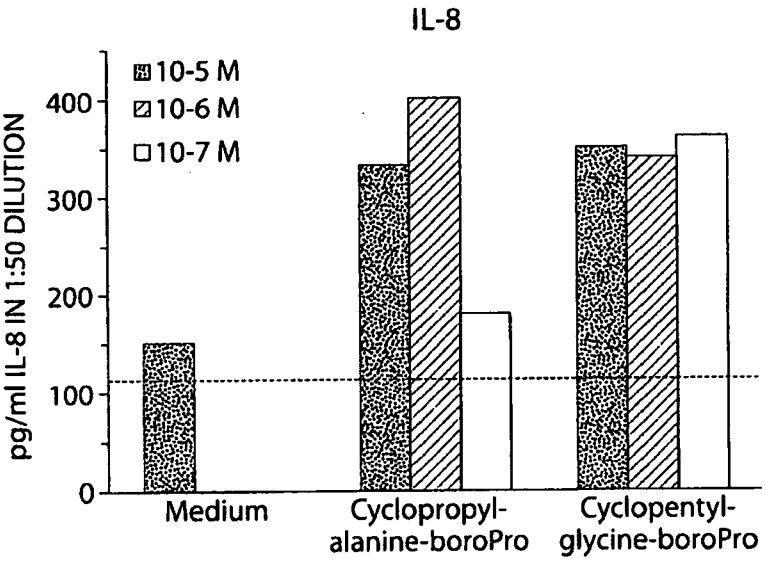

IL-8
10-5 M
10-6 M
10-7 M
pg/ml IL-8 IN 1:50 DILUTION
400
300
200
100
0
Medium
Cyclopropyl-alanine-boroPro
Cyclopentyl-glycine-boroPro

Fig. 3A

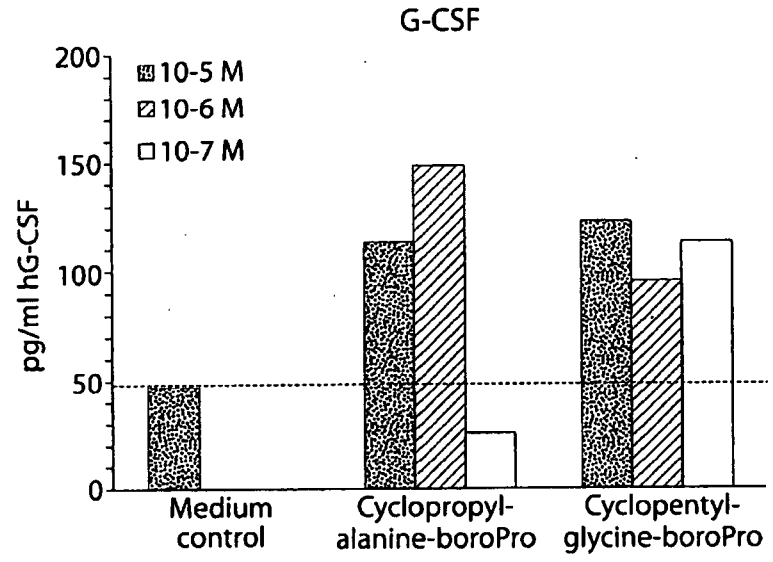

G-CSF
10-5 M
10-6 M
10-7 M
pg/ml hG-CSF
200
150
100
50
0
Medium control
Cyclopropyl-alanine-boroPro
Cyclopentyl-glycine-boroPro

Fig. 3B

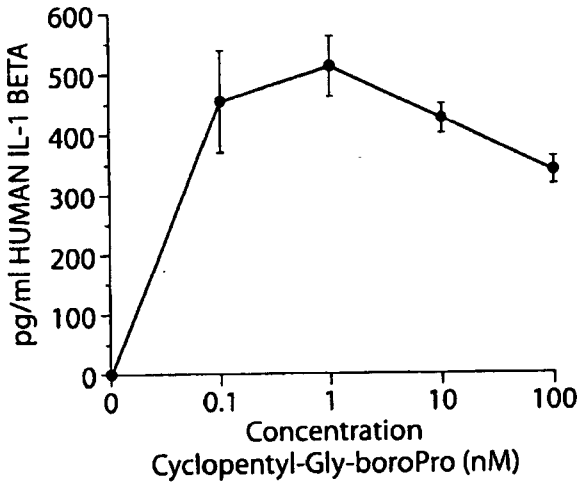

pg/ml HUMAN IL-1 BETA
600
500
400
300
200
100
0
0
0.1
1
10
100
Concentration
Cyclopentyl-Gly-boroPro (nM)

Fig. 4A

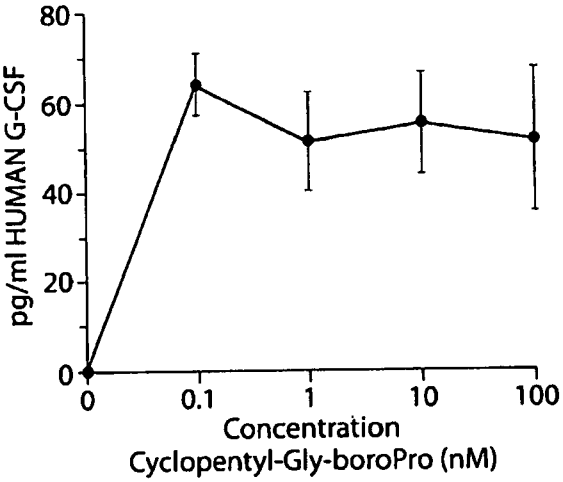

pg/ml HUMAN G-CSF
80
60
40
20
0
0
0.1
1
10
100
Concentration
Cyclopentyl-Gly-boroPro (nM)

Fig. 4B

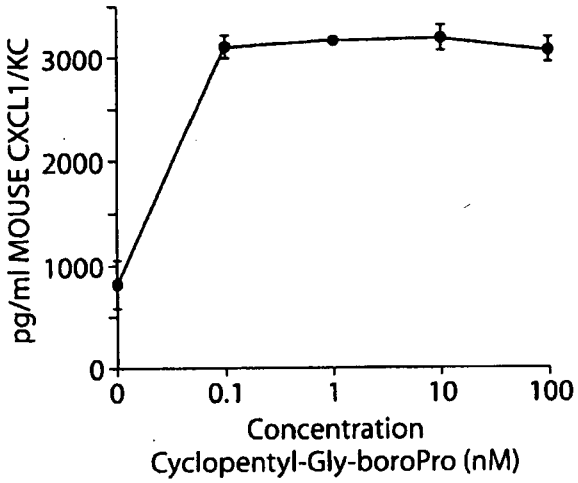

pg/ml MOUSE CXCL1/KC
3000
2000
1000
0
0
0.1
1
10
100
Concentration
Cyclopentyl-Gly-boroPro (nM)

Fig. 4C

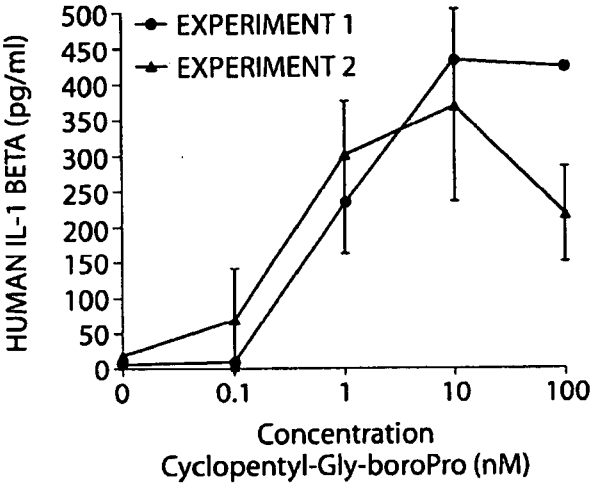

HUMAN IL-1 BETA (pg/ml)
500
450
400
350
300
250
200
150
100
50
0
EXPERIMENT 1
EXPERIMENT 2
0
0.1
1
10
100
Concentration
Cyclopentyl-Gly-boroPro (nM)

Fig. 4D

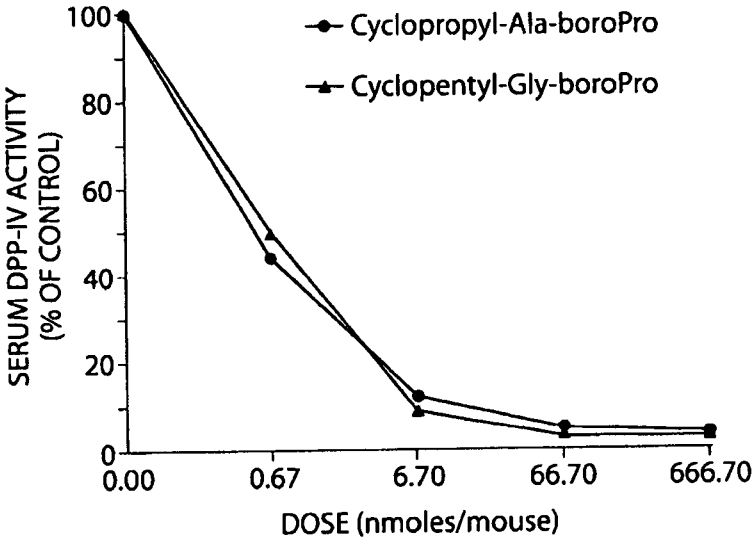

Cyclopropyl-Ala-boroPro
Cyclopentyl-Gly-boroPro
SERUM DPP-IV ACTIVITY (% OF CONTROL)
100
80
60
40
20
0
0.00
0.67
6.70
66.70
666.70
DOSE (nmoles/mouse)

Fig. 5A

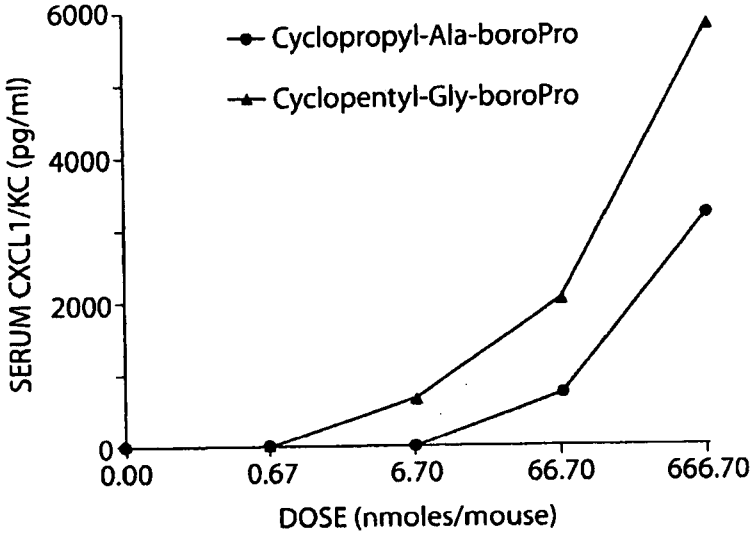

Cyclopropyl-Ala-boroPro
Cyclopentyl-Gly-boroPro
SERUM CXCL1/KC (pg/ml)
6000
4000
2000
0
0.00
0.67
6.70
66.70
666.70
DOSE (nmoles/mouse)

Fig. 5B

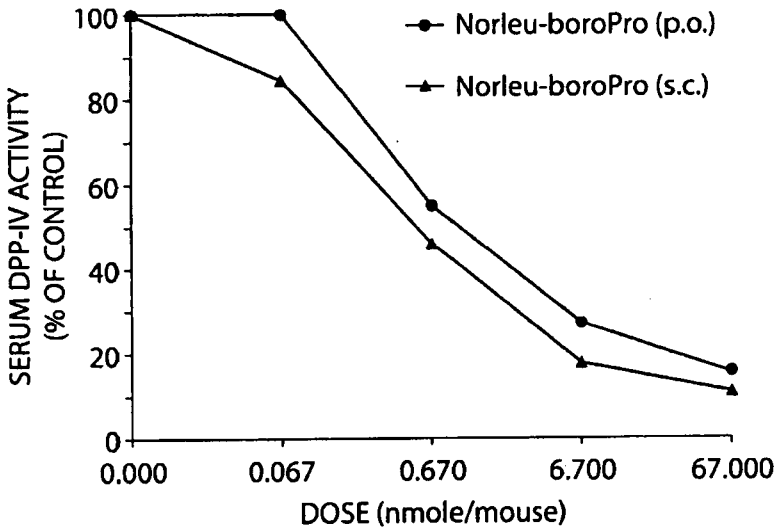

100
80
60
40
20
0
SERUM DPP-IV ACTIVITY
(% OF CONTROL)
Norleu-boroPro (p.o.)
Norleu-boroPro (s.c.)
0.000
0.067
0.670
6.700
67.000
DOSE (nmole/mouse)

Fig. 6A

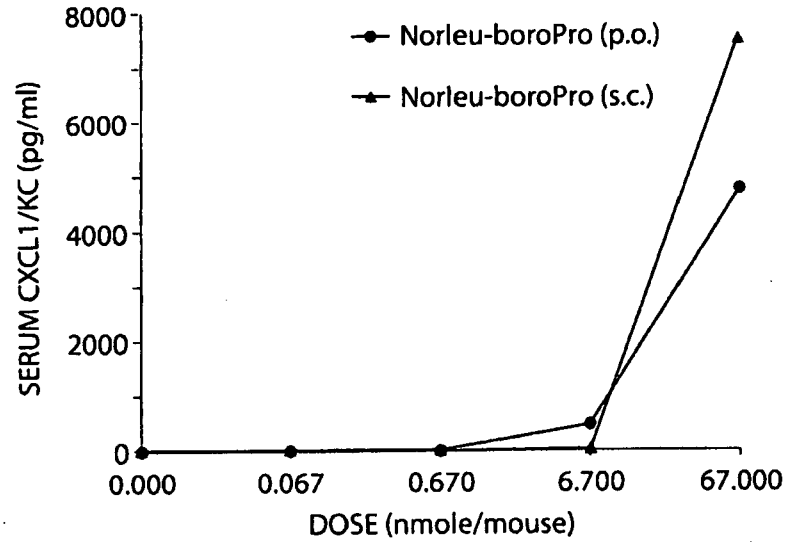

8000
6000
4000
2000
0
SERUM CXCL1/KC (pg/ml)
Norleu-boroPro (p.o.)
Norleu-boroPro (s.c.)
0.000
0.067
0.670
6.700
67.000
DOSE (nmole/mouse)

Fig. 6B

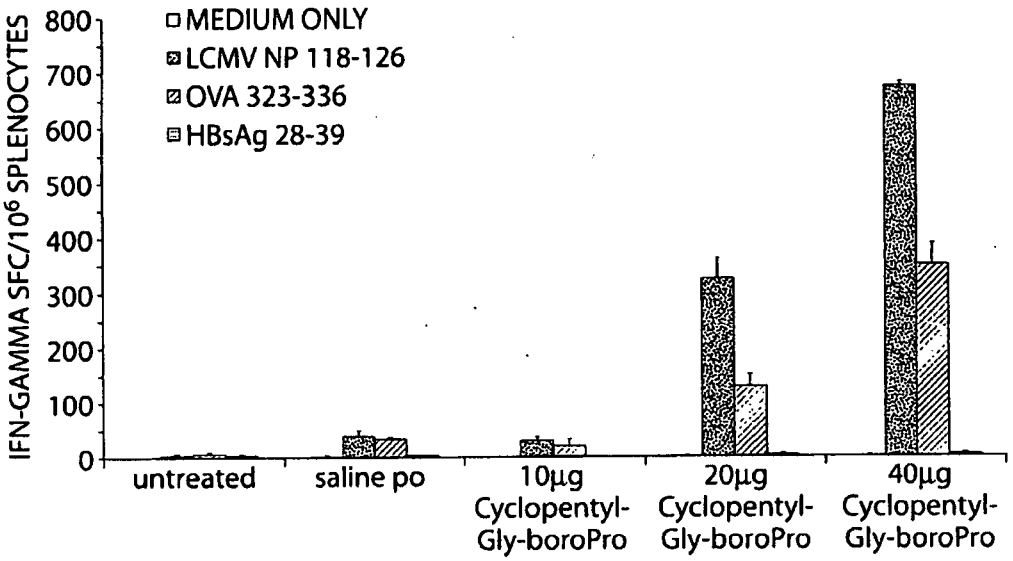

IFN-GAMMA SFC/10^6 SPLENOCYTES
800
700
600
500
400
300
200
100
0
MEDIUM ONLY
LCMV NP 118-126
OVA 323-336
HBsAg 28-39
untreated
saline po
10µg Cyclopentyl-Gly-boroPro
20µg Cyclopentyl-Gly-boroPro
40µg Cyclopentyl-Gly-boroPro

Fig. 7A

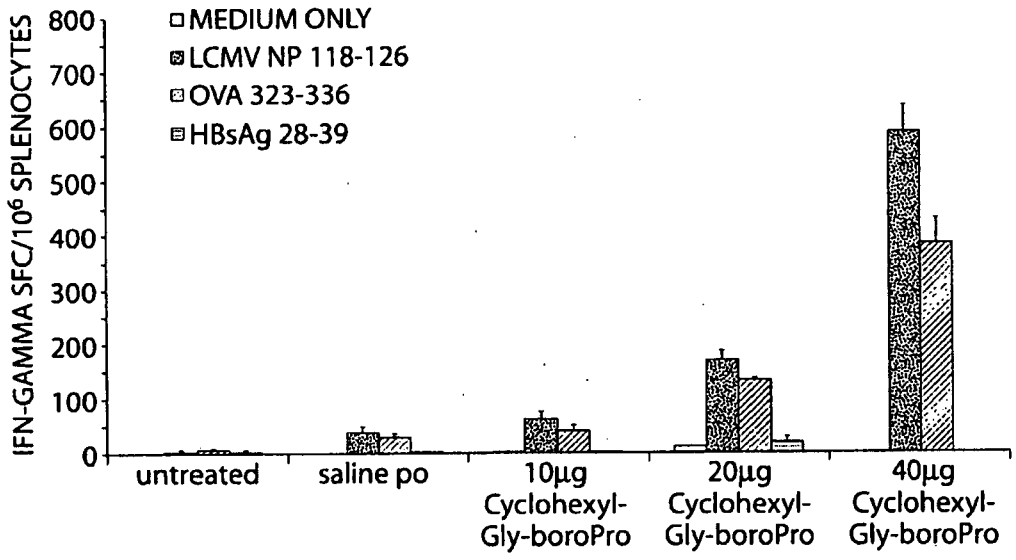

IFN-GAMMA SFC/10^6 SPLENOCYTES
800
700
600
500
400
300
200
100
0
MEDIUM ONLY
LCMV NP 118-126
OVA 323-336
HBsAg 28-39
untreated
saline po
10µg Cyclohexyl-Gly-boroPro
20µg Cyclohexyl-Gly-boroPro
40µg Cyclohexyl-Gly-boroPro

Fig. 7B

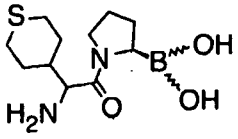
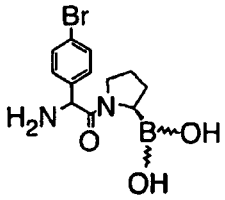
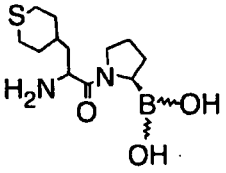
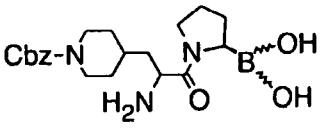
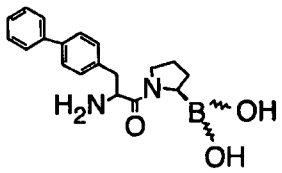
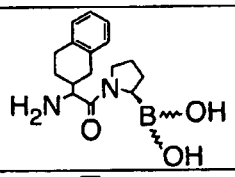
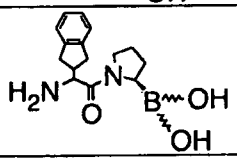
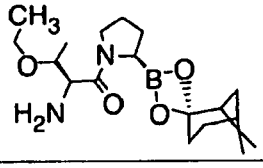
		IC_{50}(nM)			
		DPP8		DPP9	
INHIBITOR	STRUCTURE	ASSAY #1	ASSAY #3	ASSAY #2	ASSAY #4
VIf		27	17	7	23
VIIf		45	38	12	15
VIIIf		32	17	18	15
IXf		17	13	14	10.5
Xf		80	50	53	75
XIf		27	12	4.5	6
XIIe		13	4	3.5	5
XIIIf		58	61	19	18

Fig. 8

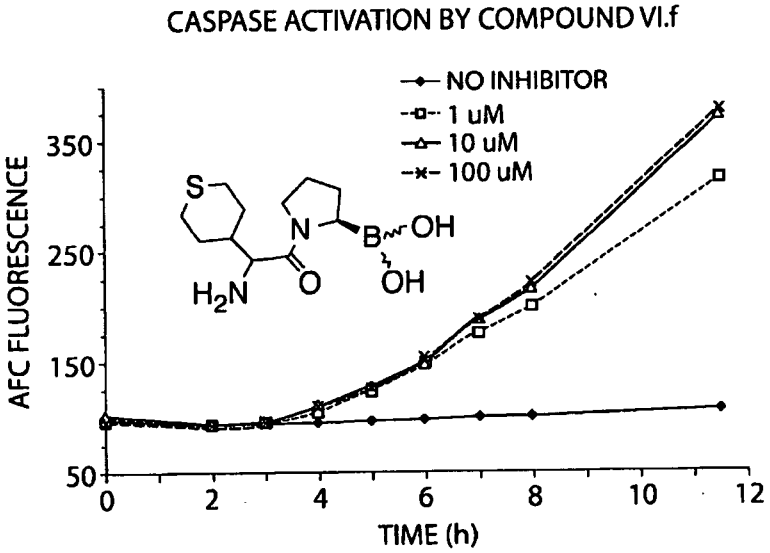

CASPASE ACTIVATION BY COMPOUND VI.f
NO INHIBITOR
1 uM
10 uM
100 uM
AFC FLUORESCENCE
350
250
150
50
0
2
4
6
8
10
12
TIME (h)
S
N
B
OH
OH
H_2N
O

Fig. 9A

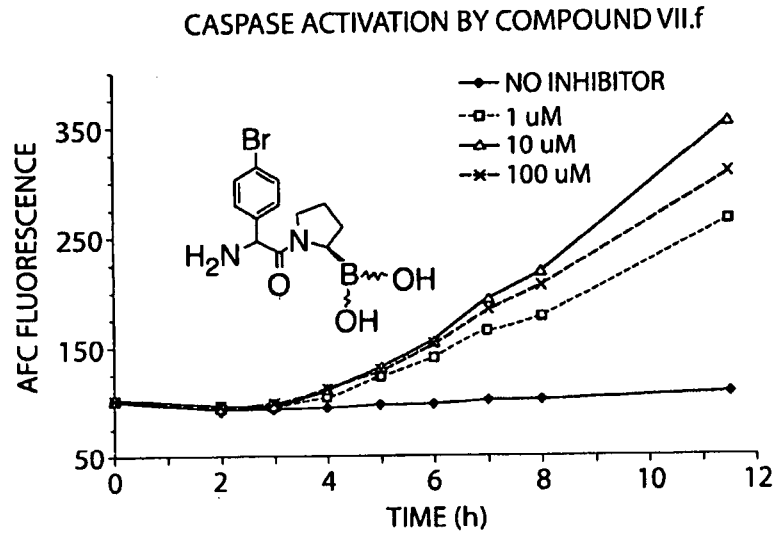

CASPASE ACTIVATION BY COMPOUND VII.f
NO INHIBITOR
1 uM
10 uM
100 uM
AFC FLUORESCENCE
350
250
150
50
0
2
4
6
8
10
12
TIME (h)
Br
H_2N
N
O
B
OH
OH

Fig. 9B

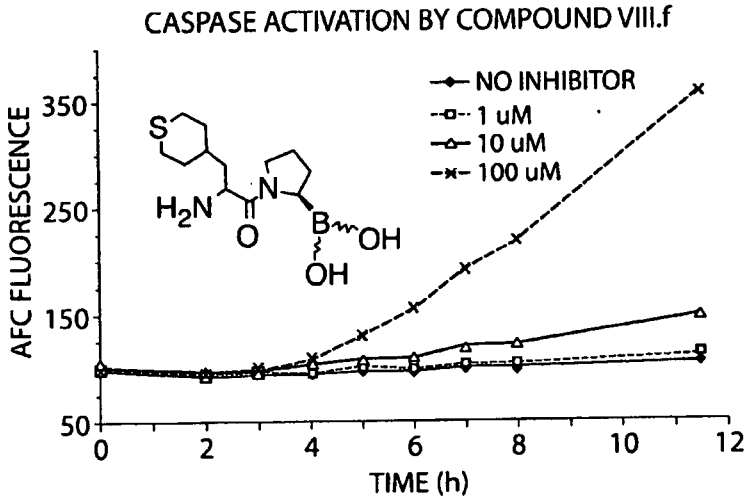

CASPASE ACTIVATION BY COMPOUND VIII.f
AFC FLUORESCENCE
350
250
150
50
0
2
4
6
8
10
12
TIME (h)
NO INHIBITOR
1 uM
10 uM
100 uM
S
N
H_2N
O
B
OH
OH

Fig. 9C

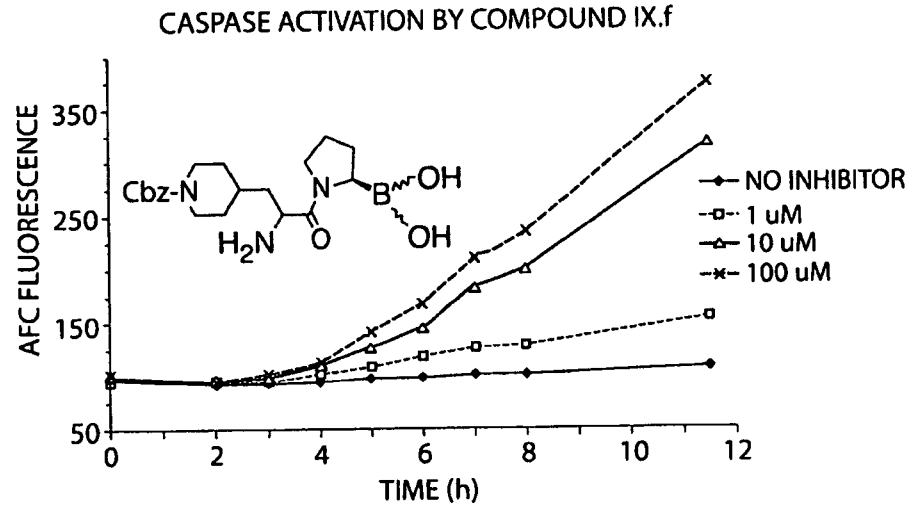

CASPASE ACTIVATION BY COMPOUND IX.f
AFC FLUORESCENCE
350
250
150
50
0
2
4
6
8
10
12
TIME (h)
NO INHIBITOR
1 uM
10 uM
100 uM
Cbz-N
N
B
OH
OH
H_2N
O

Fig. 9D

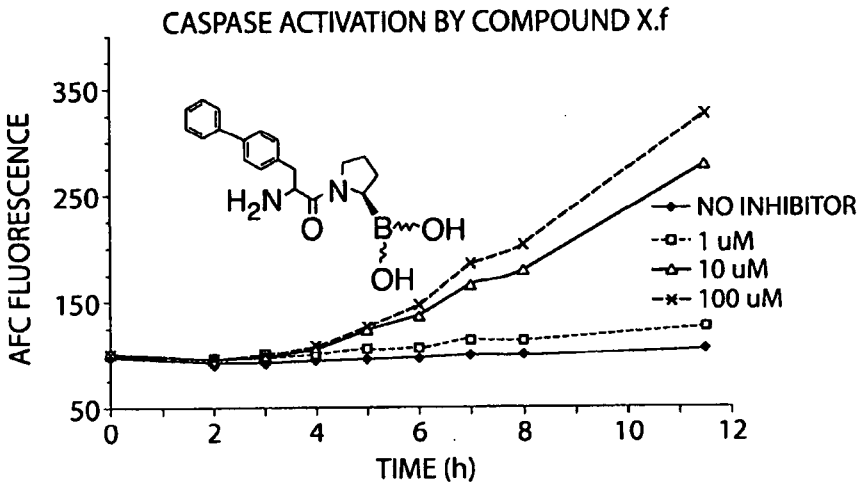

CASPASE ACTIVATION BY COMPOUND X.f
AFC FLUORESCENCE
350
250
150
50
0
2
4
6
8
10
12
TIME (h)
NO INHIBITOR
1 uM
10 uM
100 uM
H2N
O
N
B
OH
OH

Fig. 9E

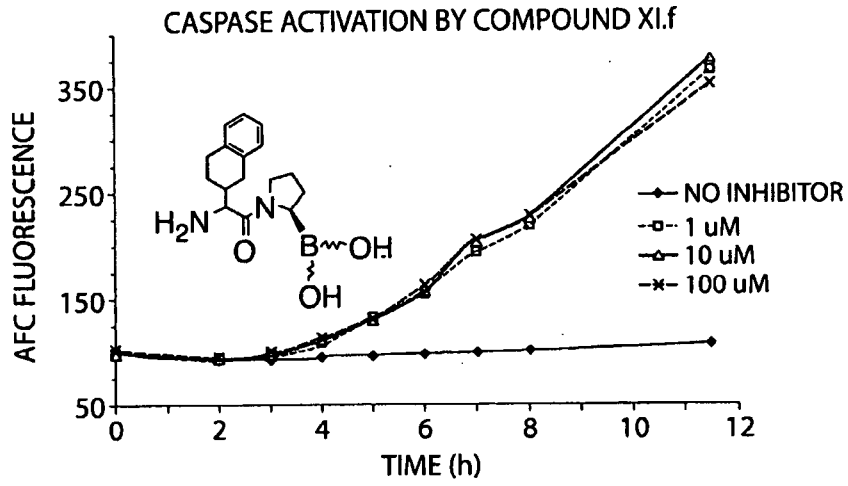

CASPASE ACTIVATION BY COMPOUND XI.f
AFC FLUORESCENCE
350
250
150
50
0
2
4
6
8
10
12
TIME (h)
NO INHIBITOR
1 uM
10 uM
100 uM
H2N
O
N
B
OH
OH

Fig. 9F

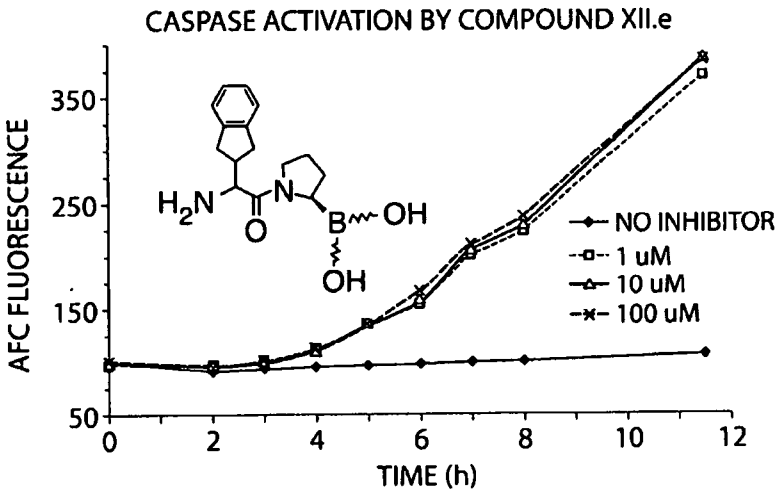

CASPASE ACTIVATION BY COMPOUND XII.e
AFC FLUORESCENCE
350
250
150
50
0
2
4
6
8
10
12
TIME (h)
NO INHIBITOR
1 uM
10 uM
100 uM

Fig. 9G

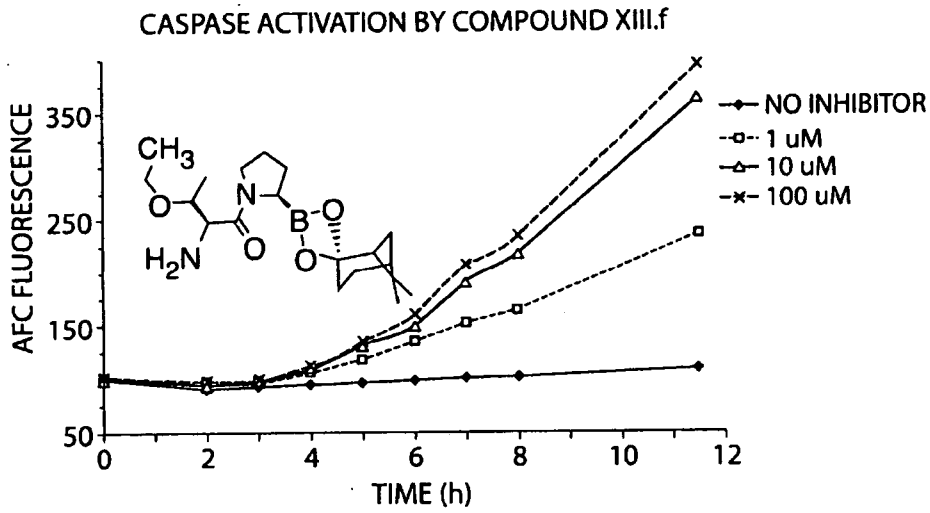

CASPASE ACTIVATION BY COMPOUND XIII.f
AFC FLUORESCENCE
350
250
150
50
0
2
4
6
8
10
12
TIME (h)
NO INHIBITOR
1 uM
10 uM
100 uM

Fig. 9H