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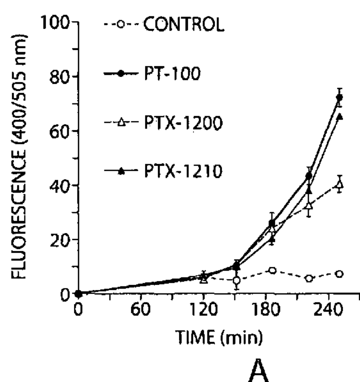
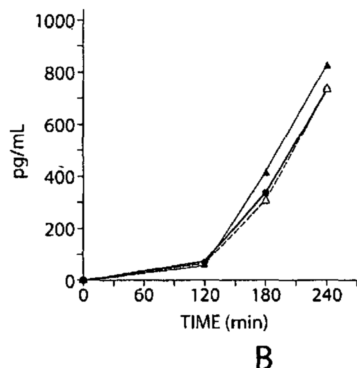
(54) Title: METHODS AND COMPOSITIONS RELATING TO IMMUNOSTIMULATION

(57) Abstract: The invention relates to methods for immunostimulation involving inhibition of DPP8 and/or 9.





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METHODS AND COMPOSITIONS RELATING TO IMMUNOSTIMULATION

Related Applications

This application claims priority to U.S. Provisional Application Serial No. 60/794371,
5 filed April 24, 2006 entitled "METHODS AND COMPOSITIONS RELATING TO IMMUNOSTIMULATION", the entire contents of which are incorporated by reference herein.

Field of the Invention

10 The invention relates to methods for inducing immunostimulation via DPP8/9 inhibition.

Background of the Invention

Abnormal cell proliferation is usually characterized by an increased rate of division
15 and in some cases uncontrolled growth. One example of a proliferative cell disorder is a tumor. In addition to posing a serious health risk in and of themselves, primary malignant tumors are particularly problematic given their tendency to invade surrounding tissues and metastasize to distant organs in the body. To date, the most frequently used methods for treating neoplasia, especially solid tumor forms of neoplasia, include surgical procedures,
20 radiation therapy, drug therapies, and combinations of the foregoing. These methods involve significant risk (e.g., of infection, death) to the patient. More importantly, the probability of eliminating all malignant cells is small particularly if the zone of malignant growth is not well defined or if the primary tumor has metastasized by the time of surgery. Achieving therapeutic doses effective for treating the cancer is often limited by the toxic side effects of
25 the anti-cancer agent on normal, healthy tissue. An ideal anti-cancer agent or therapy has tissue specificity, thereby reducing side-effects on normal (dividing) cells.

Summary of the Invention

The invention relates in part to the finding that inhibition of dipeptidyl peptidases
30 (DPP) 8 and/or 9 results in cytokine induction including IL-1 induction. The invention therefore provides methods and compositions for immune stimulation using DPP8/9 inhibitors, including selective and/or specific DPP8/9 inhibitors, as stand alone therapies or in combination with other agents.

The invention provides compositions and methods of use in the prevention and treatment of disorders that would benefit from enhanced immunostimulation. The enhanced immunostimulation may activate macrophages and other antigen presenting cells, and is therefore useful in enhancing immune responses that involve such cells including antibody dependent cell-mediated cytotoxicity and antigen presentation.

The ability of DPP8/9 inhibitors to stimulate cytokine and chemokine production endogenously is beneficial since exogenous administration of some of these factors, such as for example, IL-1, has been associated with toxicity. Production of IL-1 endogenously can be used to induce cytokines in a controlled manner, and thereby overcome toxicity problems. Although not intending to be bound by any particular mechanism, it is further proposed that induction of these cytokines from cells in vivo also indicates that feedback loops normally operating in vivo may be operative and can control cytokine levels.

The invention relates to methods and compositions for enhancing immune therapies for a number of indications, both in a therapeutic and a prophylactic sense. Immune therapies include but are not limited to passive immune therapies such as immunoglobulin administration, and active immune therapies such as vaccination with antigens alone or antigens in the context of dendritic cells. The methods are intended to treat or prevent various indications that would benefit from an enhanced immune response.

In some aspects of the invention, the DPP8/9 inhibitors are administered with an antibody or antibody fragment, with an antigen and optionally with an adjuvant, or as stand alone compositions. In some embodiments, the immune response that is stimulated is a cell-mediated immune response involving T cells, NK cells, macrophages, and the like. In other embodiments, the immune response that is stimulated is a humoral response involving B cells and antibody production. Both types of responses can co-exist in yet other embodiments. In still other embodiments, the immune response is an innate immune response, while in others it is an adaptive immune response.

In one aspect, the invention provides a method for stimulating an immune response comprising administering to a subject in need thereof a DPP8/9 inhibitor in an amount effective to stimulate an immune response.

In another aspect, the invention provides a method for stimulating an immune response in a subject comprising administering to a subject in need of immune stimulation a DPP8/9 inhibitor and an antibody or antibody fragment, in an amount effective to stimulate an immune response. Depending upon the aspect of the invention, the subject may be one in

need of immune stimulation is a subject having or at risk of developing cancer or it may be a subject having or at risk of developing an infection.

In another aspect, the invention provides a method for stimulating an immune response in a subject comprising administering to a subject in need of immune stimulation a DPP8/9 inhibitor and an antigen, in an amount effective to stimulate an antigen-specific immune response. In one embodiment, the method further comprises administering an adjuvant to the subject. The adjuvant may be alum, cholera toxin, CpG immunostimulatory nucleic acids, MPL, MPD, or QS-21. In one embodiment, the antigen is a cancer antigen.

In one embodiment, the immune response is antibody dependent cell-mediated cytoxicity. In another embodiment, the immune response is a cell-mediated immune response and/or a humoral (i.e., antibody-mediated) immune response. The immune response may be an innate immune response or an adaptive immune response, in other embodiments. In one embodiment, the immune response is an antigen specific immune response.

In one aspect, the invention provides a method of treating a subject at risk of developing an infection comprising identifying a subject at risk of developing an infectious disease, and administering an amount effective of a DPP8/9 inhibitor to the subject.

In one aspect, the invention provides a method for treating a subject having or at risk of developing a condition characterized by abnormal cell proliferation comprising administering to a subject in need thereof a DPP8/9 inhibitor in an amount effective to inhibit the condition.

In one aspect, the invention provides a method for treating a subject having or at risk of developing a condition characterized by abnormal cell proliferation comprising administering to a subject in need thereof a DPP8/9 inhibitor and a DPP4 inhibitor in an amount effective to inhibit the condition. In one embodiment, the method further comprises administering a fibroblast activation protein (FAP) inhibitor to the subject. The FAP inhibitor may be a selective or a specific inhibitor but it is not so limited.

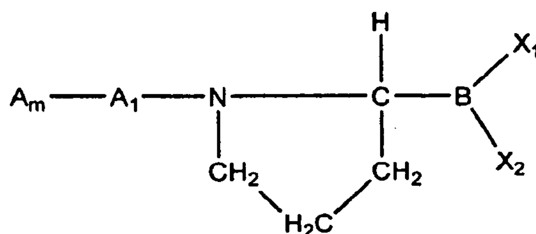
In one aspect, the invention provides a method for treating a subject having or at risk of developing a condition characterized by abnormal cell proliferation comprising administering to a subject in need thereof a DPP8/9 inhibitor and a fibroblast activation protein (FAP) inhibitor in an amount effective to inhibit the condition. In one embodiment, the method further comprises administering a DPP4 inhibitor to the subject. The DPP4 inhibitor may be a selective or a specific inhibitor but it is not so limited.

In one aspect, the invention provides a method for improving the efficacy of anti-cancer treatment comprising administering to a subject in need thereof an anti-cancer agent and a DPP8/9 inhibitor in an amount effective to improve the effect of the anti-cancer agent. In one embodiment, the anti-cancer agent is a chemotherapeutic agent. In one embodiment, the chemotherapeutic agent is docetaxel (TAXOTERE), cisplatin, gemcitabine, pemetrexed (ALIMTA), erlotinib (TARCEVA), gefitinib (IRESSA), temozolomide (TEMODAR), carboplatin, cyclophosphamide or doxorubicin. In one embodiment, the anti-cancer agent is an immunotherapeutic agent. In one embodiment, the immunotherapeutic agent is an antibody. In one embodiment, the antibody is rituximab (RITUXAN), bevacizumab (AVASTIN), cetuximab (ERBITUX), trastuzumab (HERCEPTIN), tositumomab (BEXXAR), or alemtuzumab (CAMPATH). The efficacy of antibodies or fragments thereof can be enhanced through their use in conjunction with a DPP8/9 inhibitor. Again, although not intending to be bound by any particular mechanism, it is proposed that the production of cytokines following administration of DPP8/9 inhibitors leads to the stimulation of immune cells, thereby enhancing the response mediated by the exogenously administered antibody.

In one embodiment, the subject is refractory to a prior anti-cancer treatment. In one embodiment, the prior anti-cancer treatment is different from the anti-cancer agent. In one embodiment, the prior anti-cancer treatment comprises a chemotherapeutic agent. In some embodiments, the prior anti-cancer treatment comprises an immunotherapeutic agent.

In one aspect, the invention provides a method for treating a subject having or at risk of developing a condition characterized by abnormal cell proliferation comprising administering to a subject in need thereof a DPP8/9 inhibitor in an amount effective to inhibit the condition, wherein the inhibitor is not a specific DPP4 inhibitor.

In one aspect, the invention provides a method for treating a subject having or at risk of developing a condition characterized by abnormal cell proliferation comprising administering to a subject in need thereof a DPP8/9 inhibitor in an amount effective to inhibit the condition, wherein the inhibitor is not



wherein m is an integer between 0 and 10, inclusive; A and A_1 may be L- or D-amino acid residues such that each A in A_m (i.e., where m>1) may be a different amino acid residue from every other A in A_m; the C bonded to B is in the L-configuration; and each X_1 and X_2 is, independently, a hydroxyl group or a group capable of being hydrolyzed to a hydroxyl group
5 in aqueous solution at physiological pH.

In another aspect, the invention provides a method for treating a subject having or at risk of developing an interferon (IFN)-responsive condition. The method comprises administering to a subject in need of such treatment a DPP8/9 inhibitor in an amount effective to induce a therapeutically or prophylactically effective amount of IL-1 in the subject. The
10 method may further comprise identification of a subject having or at risk of developing an IFN-responsive condition. The IFN may be IFNα, IFNα-2b, IFNβ or IFNγ, but is not so limited. The condition may be an IFNγ-responsive condition, and may be selected from the group consisting of viral infections and associated diseases, and cancer . In one embodiment, the subject is HIV positive. In one embodiment, the IFN-responsive condition is a chronic
15 infection selected from the group consisting of a chronic hepatitis B infection, chronic hepatitis C infection, chronic Epstein Barr Virus infection, and tuberculosis. Other disorders include hepatocellular carcinoma, Kaposi's Sarcoma (AIDS-related), thick primary melanomas, and regional lymph node metastases. In one embodiment, the disorder is refractive (i.e., resistant) to prior therapy (e.g., drug treatment). Thus, in one embodiment, the
20 disorder is drug resistant. In another embodiment, the disorder is multiple sclerosis. IFN-responsive conditions are not intended to be so restricted however. The method may further comprise administering to the subject a second active agent selected from the group consisting of IFNα, pegylated IFN, IFNα-2b, acyclovir, lobucavir, ganciclovir, L-deoxythymidine, clevudine, a therapeutic vaccine, phosphonoformate (PFA), ribavirin (RBV), thymosin alpha-
25 1, 2 3-dideoxy-3-fluoroguanosine (FLG), famciclovir, lamivudine, adefovir dipivoxil, entecavir, emtricitabine, and hepatitis B-specific immunoglobulin.

In a further aspect, the invention provides a method for treating a subject having or at risk of developing cancer comprising administering to a subject in need of such treatment an enzyme inhibitor selected from the group consisting of a tyrosine kinase inhibitor, a CDK
30 inhibitor, a MAP kinase inhibitor, and an EGFR inhibitor, and a DPP8/9 inhibitor in an amount effective to inhibit the cancer.

In yet one more aspect of the invention, a method is provided for treating a subject having or at risk of developing cardiovascular disease comprising administering to a subject

in need of such treatment a DPP8/9 inhibitor in an amount effective to induce an effective amount of IL-1. The method may further comprise identifying a subject in need of such treatment.

In another aspect, the invention provides a method for preventing drug resistance in a subject. The method involves administering to a subject receiving an anti-microbial agent, a DPP8/9 inhibitor in an amount effective to reduce the risk of resistance to the anti-microbial agent. In one embodiment, the subject is one having or is at risk of developing an infection. In one embodiment, the bacterial infection is a Pseudomonas infection. Other drug resistant microbes and the drugs to which they are resistant include Staphylococcus aureus (penicillin), Streptococcus pneumoniae (penicillin), gonorrhea (penicillin), and Enterococcus faecium (penicillin). In one embodiment, the anti-microbial agent is selected from the group consisting of an anti-bacterial agent, an anti-viral agent, an anti-fungal agent, and an anti-parasitic agent.

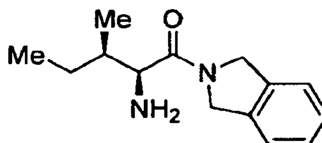
In still another aspect, the invention provides a method for shortening a vaccination course. The method may involve, in one embodiment, administering to a subject in need of immunization a DPP8/9 inhibitor in an amount effective to induce an antigen-specific immune response to a vaccine administered 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 a DPP8/9 inhibitor 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. 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). 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-1 vaccine; 5 total doses
5 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-1 vaccine; 2 total doses recommended); Td: Adult tetanus/diphtheria (2-in-1 vaccine; for use in people over age 7). In another embodiment, the DPP8/9 inhibitors can be used together with
10 oral polio vaccine.

In any of the foregoing aspects, the DPP8/9 inhibitor may be a selective or a specific DPP8/9 inhibitor. In some embodiments, the inhibitor is a selective DPP8/9 inhibitor that inhibits DPP8/9 to a greater extent than it inhibits at least DPP4. In some embodiments, the inhibitor is a selective DPP8/9 inhibitor that inhibits DPP8/9 to a greater extent than it inhibits
15 at least DPP4 and FAP.

Each of the foregoing aspects of the invention may be manifest in a number of embodiments. These embodiments are described below. It is to be understood that such embodiments apply equally to the various aspects of the invention unless stated otherwise.

Thus, in some embodiments, the DPP8/9 inhibitor is a selective inhibitor. The
20 selective DPP8/9 inhibitor may be



Lankas et al., *Diabetes* **2005**, *54*, 2988.,

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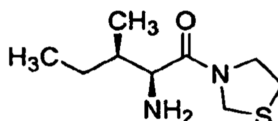

L-*allo*-isoleucyl thiazolidide **(2)**
Lankas et al., *Diabetes* **2005**, *54*, 2988.,

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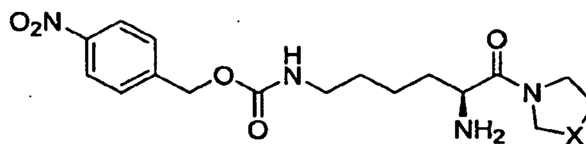

Lys[Z(NO_2)]-pyrrolidide (**8**, X = CH_2)
Lankas et al., *Diabetes* **2005**, *54*, 2988.,

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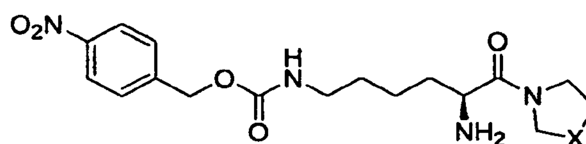

Lys[Z(NO_2)]-thiazolidide (**9**, X = S)
Lankas et al., *Diabetes* **2005**, *54*, 2988.,

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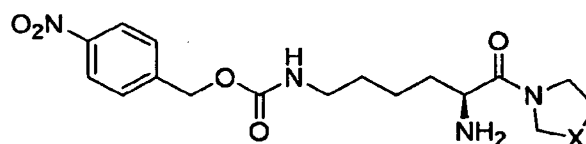

Lys[Z(NO_2)]-piperidide (**10**, X = CH_2CH_2)
Lankas et al., *Diabetes* **2005**, *54*, 2988.,

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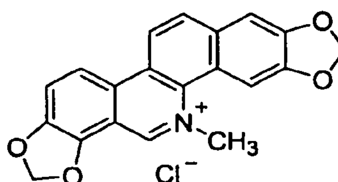

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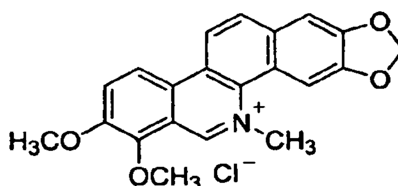
sanguinarine
Sedo et al., *Physiol. Res.* 2003, *52*, 367.,



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chelerythrine
Sedo et al., *Physiol. Res.* 2003, *52*, 367.,

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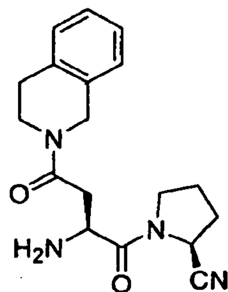

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Jiaang et al. Bioorg. Med. Chem. Lett. 15:687-691 (2005),

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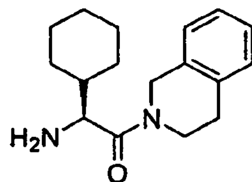

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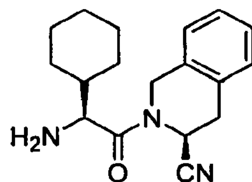
10 Jiaang et al. Bioorg. Med. Chem. Lett. 15:687-691 (2005),



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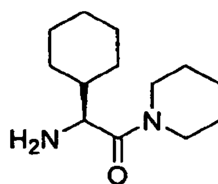
15 Jiaang et al. Bioorg. Med. Chem. Lett. 15:687-691 (2005),



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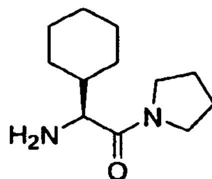
20 Jiaang et al. Bioorg. Med. Chem. Lett. 15:687-691 (2005),



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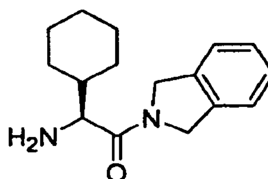

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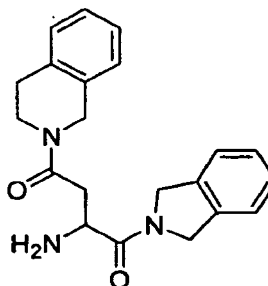
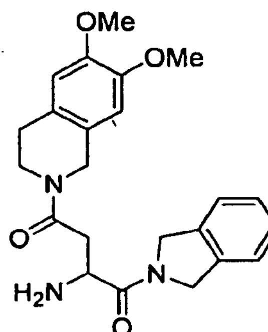
10 Jiaang et al. Bioorg. Med. Chem. Lett. 15:687-691 (2005),



15 compound 16
Jiaang et al. Bioorg. Med. Chem. Lett. 15:687-691 (2005),



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compound 17
Jiaang et al. Bioorg. Med. Chem. Lett. 15:687-691 (2005),

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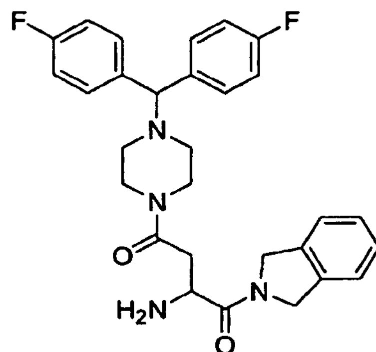

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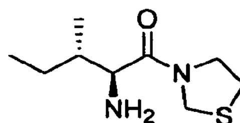
5 Jiaang et al. Bioorg. Med. Chem. Lett. 15:687-691 (2005),



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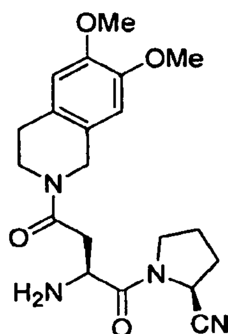
10 Lu et al. Bioorg. Med. Chem. Lett. 15:3271-3275 (2005),



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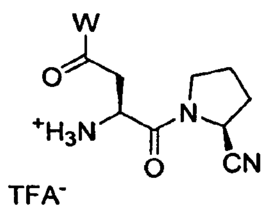

9 W = piperazine derivatives
Lu et al. Bioorg. Med. Chem. Lett. 15:3271-3275 (2005),

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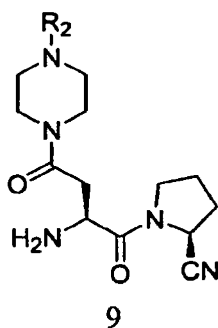

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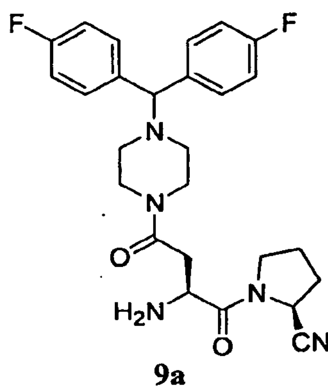

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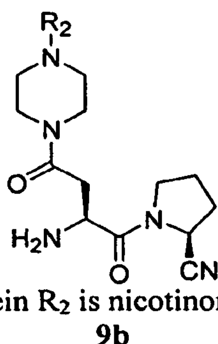
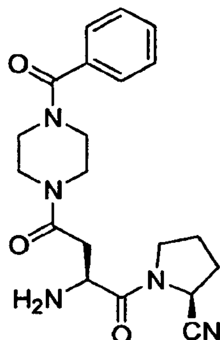


wherein R_2 is nicotinonitrile.
9b
20 Lu et al. Bioorg. Med. Chem. Lett. 15:3271-3275 (2005),



compound 9c (with R2 is benzoyl)
5 Lu et al. Bioorg. Med. Chem. Lett. 15:3271-3275 (2005),

Arg-Pro-Ala-Tyr (SEQ ID NO: 1) (see WO02/31134), Met-Phe-Ala-Tyr (SEQ ID NO: 2) (see WO02/31134), or Ile-Phe-Ala-Tyr (SEQ ID NO: 3) (WO02/31134).

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In some embodiments, the subject has a condition characterized by abnormal cell proliferation, while in others the subject is at risk of developing a condition characterized by abnormal cell proliferation. The condition may be a cancer.

The condition or the cancer may be a carcinoma such as but not limited to non-small 15 cell lung cancer, pancreatic cancer, or colorectal cancer. The condition or the cancer may be melanoma. The condition or the cancer may be a sarcoma such as but not limited to osteosarcoma or fibrosarcoma. The condition or the cancer may be a leukemia or a lymphoma. The condition or the cancer may be chronic lymphocytic leukemia. The condition or the cancer may be refractory to a prior treatment. The condition or the cancer 20 may be a primary tumor. In some embodiments, the cancer expresses normal or below-normal levels of DPP8 and/or 9 or does not express DPP8 and/or 9.

In some embodiments, the method further comprises administering to the subject a second therapeutic agent that is an anti-cancer agent. The anti-cancer agent may be a chemotherapeutic agent such as but not limited to docetaxel, cisplatin, gemcitabine, 25 pemetrexed (ALIMTA), erlotinib (TARCEVA), gefitinib (IRESSA), temozolomide (TEMODAR), carboplatin, cyclophosphamide or doxorubicin.

In some embodiments, the method further comprises administering to the subject a second therapeutic agent that is an immunotherapeutic agent. The immunotherapeutic agent may be an antibody or an antibody fragment including but not limited to rituximab

(RITUXAN), bevacizumab (AVASTIN), cetuximab (ERBITUX), trastuzumab (HERCEPTIN), tositumomab (BEXXAR), or alemtuzumab (CAMPATH).

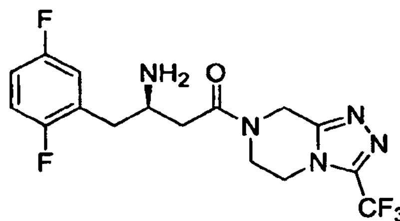
The immunotherapeutic agent may an antigen such as a cancer antigen or a microbial antigen such as a bacterial antigen, a viral antigen, a fungal antigen, a parasitic antigen or a mycobacterial antigen. The cancer antigen may be MART-1/Melan-A, gp100, adenosine deaminase-binding protein (ADAbp), FAP, cyclophilin b, colorectal associated antigen (CRC)--C017-1A/GA733, carcinoembryonic antigen (CEA), CAP-1, CAP-2, etv6, AML1, prostate specific antigen (PSA), PSA-1, PSA-2, PSA-3, prostate-specific membrane antigen (PSMA), T-cell receptor/CD3-zeta chain, and CD20. The cancer antigen may also be selected from the group consisting of MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE-C1, MAGE-C2, MAGE-C3, MAGE-C4, MAGE-C5). In still another embodiment, the cancer antigen is selected from the group consisting of GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, GAGE-9. And in yet a further embodiment, the cancer antigen is selected from the group consisting of BAGE, RAGE, LAGE-1, NAG, GnT-V, MUM-1, CDK4, tyrosinase, p53, MUC family, HER2/neu, p21ras, RCAS1, α-fetoprotein, E-cadherin, α-catenin, β-catenin, γ-catenin, p120ctn, gp100^{Pmel117}, PRAME, NY-ESO-1, cdc27, adenomatous polyposis coli protein (APC), fodrin, Connexin 37, Ig-idiotype, p15, gp75, GM2 ganglioside, GD2 ganglioside, human papilloma virus proteins, Smad family of tumor antigens, lmp-1, P1A, EBV-encoded nuclear antigen (EBNA)-1, brain glycogen phosphorylase, SSX-1, SSX-2 (HOM-MEL-40), SSX-1, SSX-4, SSX-5, SCP-1 and CT-7, CD20, and c-erbB-2.

In certain embodiments, the methods may further comprise treating the subject with a therapy selected from the group consisting of surgery, radiation and chemotherapy.

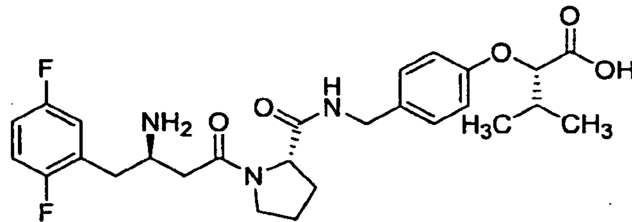
In one embodiment, the DPP8/9 inhibitor and the antigen (or the antibody) are administered prior to treating the subject with a therapy selected from the group consisting of surgery, radiation and chemotherapy. In another embodiment, the DPP8/9 inhibitor and the antigen (or antibody) are administered after treating the subject with a therapy selected from the group consisting of surgery, radiation and chemotherapy. In yet another embodiment, the DPP8/9 inhibitor and the antigen (or antibody) are administered before and after treating the subject with a therapy selected from the group consisting of surgery, radiation and chemotherapy.

In some embodiments, the method further comprises administering to the subject a second therapeutic agent that is a cytokine. The cytokine may be G-CSF or GM-CSF, or it may be M-CSF.

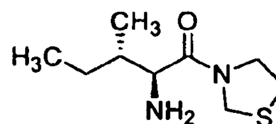
In some embodiments, the method further comprises administering to the subject a DPP4 inhibitor. The DPP4 inhibitor may be a selective inhibitor or a specific inhibitor, although it is not so limited. The DPP4 inhibitor may be a selective DPP4 inhibitor such as



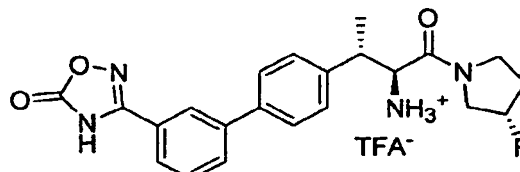
Lankas et al., *Diabetes* **2005**, *54*, 2988.,



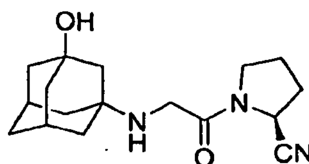
Lankas et al., *Diabetes* **2005**, *54*, 2988.,



L-*threo*-isoleucyl thiazolidide
Lankas et al., *Diabetes* **2005**, *54*, 2988.,



5-oxo-1,2,4-oxadiazole
Xu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15(10)*, 2533.,



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I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

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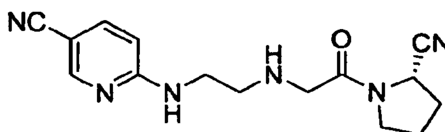
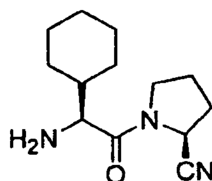


2, DPP728
10 I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,



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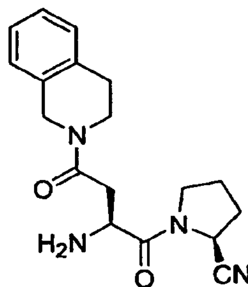
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,



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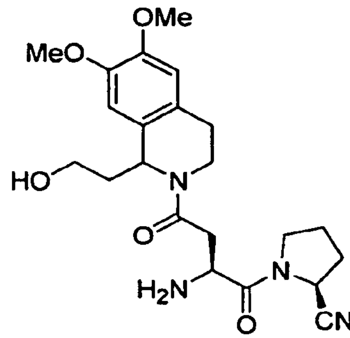

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I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

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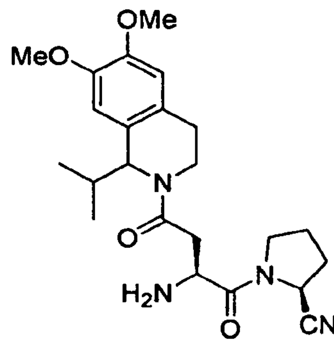

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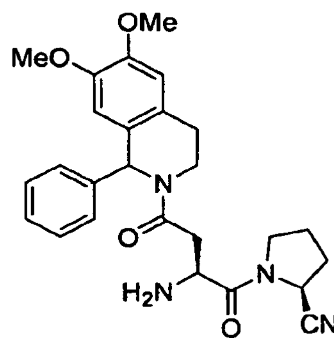

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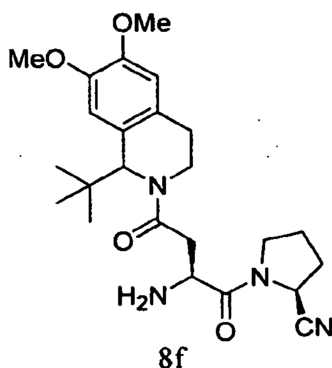

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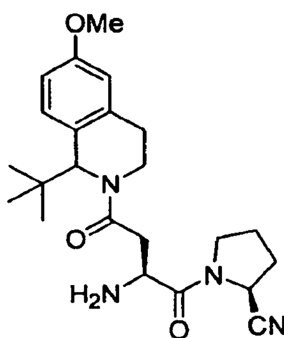

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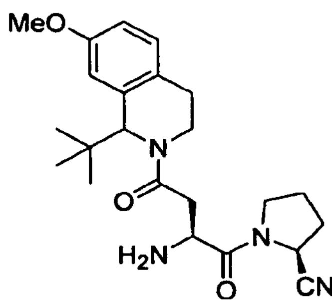

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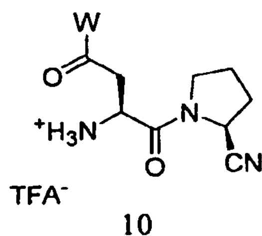

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10 W = benzyl derivatives
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

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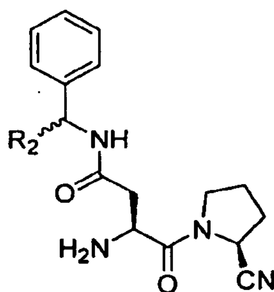

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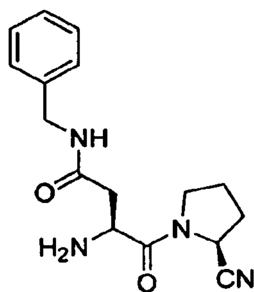

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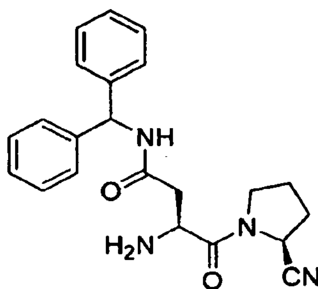
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,



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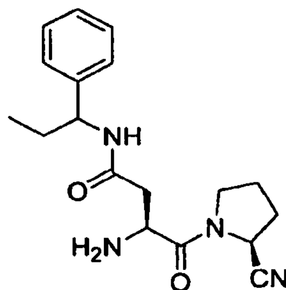
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,



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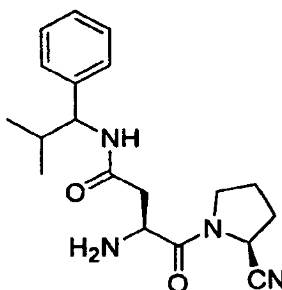

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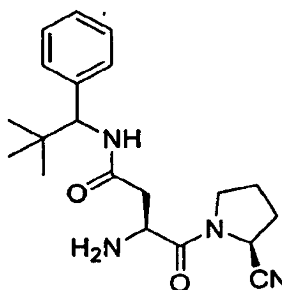
10 I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,



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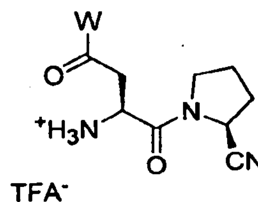

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11 W = Phenethylamine derivatives
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

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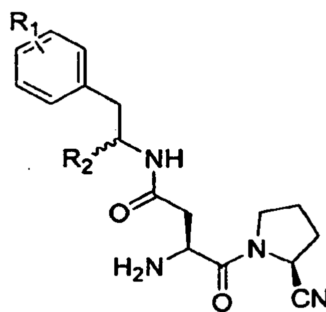

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I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

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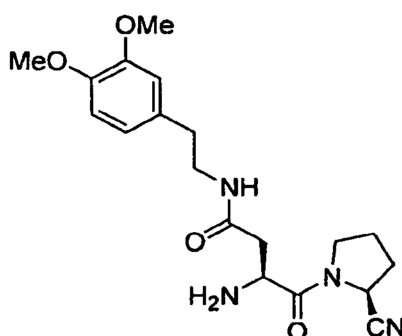

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I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

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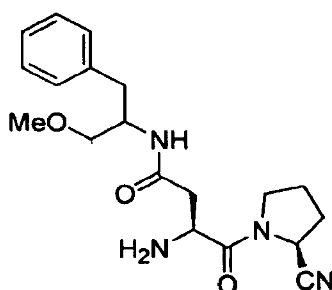

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I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271., or

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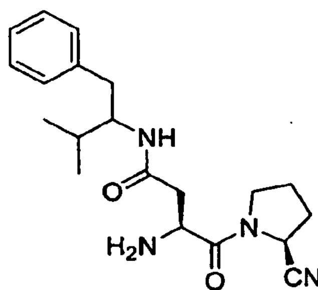

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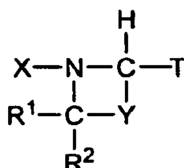
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271..

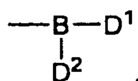
In some embodiments, the DPP4 inhibitor has an IC_{50} for DPP8/9 that is at least 3 fold higher than its IC_{50} for DPP4. In some embodiments, the DPP4 inhibitor has an IC_{50} for DPP8/9 that is at least 5 fold higher than its IC_{50} for DPP4.

5 In some embodiments, the selective DPP8/9 inhibitor is administered prior to the DPP4 inhibitor. In some embodiments, the selective DPP8/9 inhibitor and the DPP4 inhibitor are administered to the subject substantially simultaneously. In some embodiments, the selective DPP8/9 inhibitor and the DPP4 inhibitor are administered to the subject in an alternating manner.

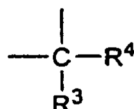
10 In some embodiments, the method further comprises administering to the subject a boroProline compound having a structure of



where T is a boronate group of the formula

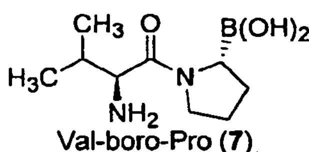


15 where each D^1 and D^2, independently, is a hydroxyl group or a group which is capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH; X comprises an amino acid or a peptide which mimics the site of a substrate recognized by a post-prolyl cleaving enzyme; Y is



20 and each R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8 is a hydrogen.

In some embodiments, the boroProline compound is



25 Val-boro-Pro (7).

In some embodiments, the selective DPP8/9 inhibitor is administered prior to the boroProline compound. In some embodiments, the selective DPP8/9 inhibitor and the

boroProline compound are administered to the subject substantially simultaneously. In some embodiments, the selective DPP8/9 inhibitor and the boroProline compound are administered to the subject in an alternating manner.

In some embodiments, the subject has or is at risk of developing an infection such as a bacterial infection, a viral infection, a fungal infection, a parasitic infection or a mycobacterial infection. In some embodiments, the method further comprises administering to the subject an anti-microbial agent such as an anti-bacterial agent, an anti-viral agent, an anti-fungal agent, an anti-parasitic agent, or an anti-mycobacterial agent.

The bacterial infection may be an E. coli infection, a Staphylococcal infection, a Streptococcal infection, a Pseudomonas infection, Clostridium difficile infection, Legionella infection, Pneumococcus infection, Haemophilus infection, Klebsiella infection, Enterobacter infection, Citrobacter infection, Neisseria infection, Shigella infection, Salmonella infection, Listeria infection, Pasteurella infection, Streptobacillus infection, Spirillum infection, Treponema infection, Actinomyces infection, Borrelia infection, Corynebacterium infection, Nocardia infection, Gardnerella infection, Campylobacter infection, Spirochaeta infection, Proteus infection, Bacteriodes infection, H. pylori infection, or anthrax infection.

The mycobacterial infection may be tuberculosis or leprosy respectively caused by the M. tuberculosis and M. leprae species, but is not so limited.

The viral infection may be a Herpes simplex virus 1 infection, a Herpes simplex virus 2 infection, cytomegalovirus infection, hepatitis A virus infection, hepatitis B virus infection, hepatitis C virus infection, human papilloma virus infection, Epstein Barr virus infection, rotavirus infection, adenovirus infection, influenza A virus infection, respiratory syncytial virus infection, varicella-zoster virus infections, small pox infection, monkey pox infection, SARS infection or avian flu infection. In one embodiment, the viral infection is not an HIV infection.

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

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

In one embodiment, the antigen is a microbial antigen. As used herein, a microbial antigen is an antigen derived from an infectious pathogen, and may include the entire pathogen. The antigen may be peptide, lipid, or carbohydrate in nature, but it is not so limited.

5 In one embodiment, the microbial antigen is selected from the group consisting of a bacterial antigen, a mycobacterial antigen, a viral antigen, a fungal antigen, and a parasitic antigen.

In one embodiment, the bacterial, viral, fungal, parasitic and mycobacterial antigen may be derived from any of the bacterial, viral, fungal, parasitic and mycobacterial species 10 recited herein, respectively.

In some embodiments, the antibody or antibody fragment is conjugated (covalently or otherwise) to a toxin derived from plant, fungus, or bacteria. 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 and Pseudomonas 15 exotoxin, but is not so limited.

In some embodiments, the antibody or antibody fragment is conjugated to a chemotherapeutic agent, a radioisotope or a cytotoxin. The chemotherapeutic agent 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.

20 When administered with another agent, the DPP8/9 inhibitors may be formulated together or separately from the other agent, depending on the administration regimen, route, and like.

The invention further contemplates compositions comprising one or more of the foregoing agents including combinations of DPP8/9 inhibitors, DPP4 inhibitors, FAP 25 inhibitors, antibodies, antigens, adjuvants, cytokines, anti-bacterial agents, anti-viral agents, anti-fungal agents, anti-parasitic agents, anti-mycobacterial agents, and the like.

The invention provides in yet another aspect a composition comprising an effective amount of a DPP8/9 inhibitor and an antibody or antibody fragment. In one embodiment, the composition further comprises a pharmaceutically acceptable carrier.

30 In one embodiment, the effective amount is an amount to stimulate antibody dependent cell-mediated cytoxicity. In another embodiment, the effective amount is an amount to treat or prevent cancer. In still another embodiment, the effective amount is an amount to treat or prevent an infectious disease.

In one embodiment, the antibody or antibody fragment is an antibody, and it can be selected from the group listed above.

In another aspect, the invention provides a composition comprising an effective amount of a DPP8/9 inhibitor and a cancer antigen. In one embodiment, the effective amount is an amount to treat or prevent cancer. In this and other aspects of the invention, the cancer antigen may be a peptide antigen, or a lipid antigen, but it is not so limited. The cancer antigen can be selected from the groups recited above.

The invention also contemplates kits comprising these agents, compositions and/or combinations. The compositions may be provided in a housing such as a container, a box, or a bag. The housing may also contain instructions for use of the composition either thereon or therein. The instructions for use indicate how the contents of the housing are to be used, including timing and dose of administrations.

These and other aspects and embodiments will be described in greater detail herein.

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. This invention is not limited in its application to the details of construction and/or the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways.

The phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. The use of "including", "comprising", or "having", "containing", "involving", and variations thereof herein, is meant to encompass the items listed thereafter and equivalents thereof as well as additional items.

Brief Description of the Figures

FIG. 1 demonstrates activation of caspase-1 and induction of IL-1β by PT-100 (talabostat, L-valinyl-L-boroproline) and the DPP8/9 selective inhibitors PTX-1200 (cyclohexyl glycine-isoindoline) and PTX-1210 (1-Bis(4-fluorophenyl)methyl piperazine/aspartate/isoindoline) in cultures of PMA-differentiated THP-1 human monocytic cells. After overnight differentiation with PMA, THP-1 cells were incubated (37°C) in ≤3kDa factor in multiwell plates with PT-100, PTX-1200 or PTX-1210, each at a concentration of 10 μM, or without addition of a compound (control). Caspase-1 activity was measured by the

Ac-WEHD-AFC fluorogenic assay (A) and secreted IL-1β by ELISA (B) at the time points indicated on the abscissae.

FIG. 2 demonstrates activation of caspase-1 by PT-100 (talabostat, L-valinyl-L-boroproline) and the DPP8/9 selective inhibitor PTX-1210 (1-Bis(4-fluorophenyl)methyl piperazine/aspartate/isoindoline) in cultures of undifferentiated THP-1 human monocytic cells. THP-1 cells were incubated (37°C) in multiwell plates with PT-100, or PTX-1210, each at a concentration of 10 μM, or without addition of a compound (control). Caspase-1 activity was measured by the Ac-WEHD-AFC fluorogenic assay at the time points indicated on the abscissae.

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 the finding that inhibition of dipeptidyl peptidase 8 and/or 9 (DPP8/9) results in induction of IL-1 and various other cytokines and chemokines. Although not intending to be bound by any particular mechanism, it is now believed that DPP8/9 inhibition results in activation of caspase I which in turn results in at least IL-1beta secretion. This is followed by the upregulation of a number of cytokines and chemokines for example by autocrine and/or paracrine pathways. These cytokines and chemokines include but are not limited to IL-1alpha, IL-6, G-CSF, MCP-1/CCL2, MCP-2/CCL8, MCP-3/MARC/CCL7, MCP-5/CCL12, MRP-1/CCL6, JBE, MIP-1alpha/CCL3, MIP-1beta/CCL4, MIP-2/CXCL2, MIG/CXCL9, JE, TARC/CCL17, Lymphotactin/XCL1, IL-8/KC/CXCL1, ENA78/CXCL5, LIX, eotaxin/CCL11, IP-10/CXCL10, MDC/CCL22, IFNbeta, and thrombospondin. In addition to stimulating cytokine and chemokine production, IL-1beta may also upregulate expression of endothelial adhesion molecules which can in turn facilitate migration of neutrophils. Thus, for example, DPP8/9 inhibitors are able to cause the release of neutrophils from the bone marrow via the induction of IL-1beta.

The invention therefore provides methods for stimulating immune responses by inhibiting DPP8/9. These methods can form the basis of various therapies that are immune responsive including but not limited to cancer and infectious disease. The invention contemplates the use of DPP8/9 inhibitors alone or in combination with other DPP inhibitors or other secondary agents.

In various aspects of the invention, the methods are intended to stimulate an immune response in a subject. The immune response may be a cell-mediated immune response and/or

a humoral (i.e., antibody-mediated) immune response. The immune response may be an innate immune response or an adaptive immune response. In one embodiment, the immune response is an antigen specific immune response. The DPP8/9 inhibitors can be used to stimulate or enhance antibody dependent cell-mediated cytoxicity (ADCC), particularly when administered with an antibody or antibody fragment.

The immunostimulatory DPP8/9 inhibitors of the invention can be used in a number of conditions as described in greater detail herein. These conditions are generally those that benefit from immunostimulation, rather than those that require immunosuppression (e.g., autoimmune diseases, allograft transplant rejection, etc.).

The methods of the invention use DPP8/9 inhibitors. As used herein, a DPP8/9 inhibitor is a compound that inhibits the enzymatic activity of DPP8 and/or DPP9. Various methods provided herein employ selective or specific DPP8/9 inhibitors. As used herein, a selective DPP8/9 inhibitor is a compound that inhibits DPP8/9 (i.e., DPP8 and/or DPP9) to a greater extent than it does at least one (including two or three) other post-prolyl cleaving enzyme. For example, a selective DPP8/9 inhibitor inhibits DPP8/9 to a greater extent than it does DPP4, or fibroblast activation protein (FAP), or PEP, or DPP2. The selective DPP8/9 inhibitor may inhibit DPP8/9 to a greater extent than it does two or three other post-prolyl cleaving enzymes. For example, a selective DPP8/9 inhibitor may inhibit DPP8/9 to a greater extent than it does DPP4 and FAP, or DPP4 and PEP, or FAP and PEP, or DPP2 and DPP4, etc. As another example, a selective DPP8/9 inhibitor may inhibit DPP8/9 to a greater extent than it does DPP4, FAP and PEP, or DPP2, DPP4 and FAP, or DPP2, FAP and PEP, etc. As used herein, a specific DPP8/9 inhibitor is a compound that inhibits DPP8/9 to a greater extent than it does at least non-DPP8/9 post-prolyl cleaving enzymes DPP2, DPP4, FAP and PEP. Enzyme inhibition may be measured as the concentration of compound required to inhibit enzymatic activity in vitro by 50%. This measure is referred to herein as the IC_{50}, and it is usually expressed as a submolar value (e.g., nM). Accordingly, a selective DPP8/9 inhibitor has a lower IC_{50} for DPP8/9 than for at least one non-DPP8/9 post-prolyl cleaving enzyme, including DPP4 and fibroblast activation protein (FAP). A specific DPP8/9 inhibitor has a lower IC_{50} for DPP8/9 than for non-DPP8/9 post-prolyl cleaving enzymes DPP2, DPP4, FAP and PEP.

In some instances, the selective DPP8/9 inhibitor has an IC_{50} for DPP8/9 that is at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 7-fold, at least 10-fold, at

least 50-fold, at least 100-fold, or at least 1000 fold less than the IC_{50} for at least one other post-prolyl cleaving enzyme.

In some instances, the specific DPP8/9 inhibitor has an IC_{50} for DPP8/9 that is at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 7-fold, at least 10-fold, at least 50-fold, at least 100-fold, or at least 1000 fold less than the IC_{50} for post-prolyl cleaving enzymes DPP2, DPP4, FAP and PEP.

The invention contemplates the use of DPP4 inhibitors, including selective and specific DPP4 inhibitors. As used herein, a DPP4 inhibitor is a compound that inhibits the enzymatic activity of DPP4. As used herein, a selective DPP4 inhibitor is a compound that has a lower IC_{50} for DPP4 than for at least one other post-prolyl cleaving enzyme. The definition is similar to that for selective DPP8/9 inhibitors as taught herein. As used herein, a specific DPP4 inhibitor is a compound that has a lower IC_{50} for DPP4 than for post-prolyl cleaving enzymes DPP2, DPP8, DPP9, FAP and PEP. The invention also contemplates the use of FAP inhibitors including selective and specific FAP inhibitors. As used herein, a FAP inhibitor is a compound that inhibits the enzymatic activity of FAP. As used herein, a selective FAP inhibitor is a compound that has a lower IC_{50} for DPP4 than for at least one other post-prolyl cleaving enzyme. The definition is similar to that for selective DPP8/9 inhibitors as taught herein. As used herein, a specific FAP inhibitor is a compound that has a lower IC_{50} for FAP than for post-prolyl cleaving enzymes DPP2, DPP4, DPP8, DPP9 and PEP. The invention further contemplates the use of PEP inhibitors including selective and specific PEP inhibitors. As used herein, a PEP inhibitor is a compound that inhibits the enzymatic activity of PEP. As used herein, a selective PEP inhibitor is a compound that has a lower IC_{50} for PEP than for at least one other post-prolyl cleaving enzyme. The definition is similar to that for selective DPP8/9 inhibitors as taught herein. As used herein, a specific PEP inhibitor is a compound that has a lower IC_{50} for PEP than for other post-prolyl cleaving enzymes.

It is to be understood that in embodiments involving the use and/or administration of a DPP8/9 inhibitor and a DPP4 inhibitor, it is intended that these inhibitors are two distinct compounds, rather than one and the same compound. Similarly, in embodiments involving the use and/or administration of a DPP8/9 inhibitor and a FAP inhibitor, it is intended that these inhibitors are two distinct compounds, rather than one and the same compound, and in embodiments involving the use and/or administration of a DPP8/9 inhibitor, a DPP4 inhibitor and a FAP inhibitor, it is intended that these inhibitors are three distinct compounds, rather

than one and the same compound. This meaning is intended for all combinations of inhibitors described herein.

It is also to be understood that where an inhibitor is stated, selective and/or specific inhibitors are included but may not be recited for the sake of brevity, unless stated otherwise.

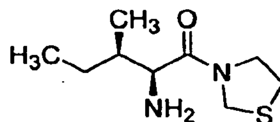
Post-prolyl cleaving enzymes are enzymes that cleave peptides (including proteins) at the carboxy terminal of preferably a proline residue. Post-prolyl cleaving enzymes can have endopeptidase and/or exopeptidase activity. Post-prolyl cleaving enzymes with exopeptidase activity include dipeptidyl peptidases. Dipeptidyl peptidases (DPPs or DPs) are enzymes that cleave dipeptides from the amino terminus of a peptide (e.g., a protein) provided that the penultimate residue is a proline (and sometimes an alanine). These enzymes cleave at the carboxy side of the penultimate residue.

Post-prolyl cleaving enzymes include but are not limited to DPP2, DPP4, DPP8, DPP9, fibroblast activation protein (FAP) and prolylendopeptidase (PEP). DPP2, DPP4, DPP8 and DPP9 have exopeptidase activities. PEP has endopeptidase activity. FAP has both exo- and endopeptidase activity. All of these enzymes are known in the art.

Assays for determining IC_{50} of a compound with respect to one or more post-prolyl cleaving enzymes are described in the Examples, as well as in U.S. Pat. No. 6,890,904, issued on May 10, 2005, particularly in Example 1, and in U.S. Published Application 2006-0063719 A1, published on March 23, 2006, and such assay descriptions are incorporated by reference herein.

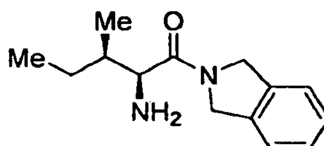
Examples of DPP8/9 inhibitors including selective and specific DPP8/9 inhibitors are as follows:

(a)



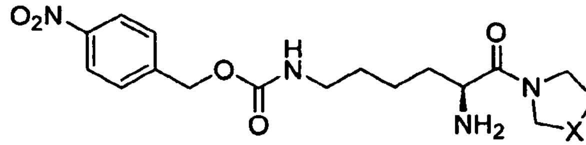
L-*allo*-isoleucyl thiazolidide **(2)**
Lankas et al., *Diabetes* **2005**, *54*, 2988.,

(b)



4
Lankas et al., *Diabetes* **2005**, *54*, 2988.,

(c)



Lys[Z(NO_2)]-pyrrolidide (**8**, X = CH_2)
Lankas et al., *Diabetes* **2005**, *54*, 2988.,

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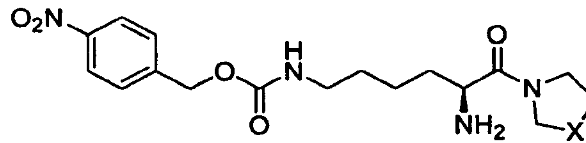
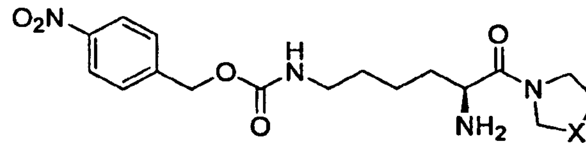
(d)



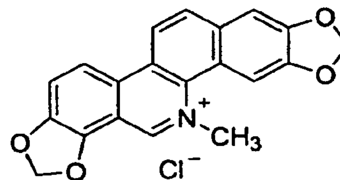
Lys[Z(NO_2)]-thiazolidide (**9**, X = S)
10 Lankas et al., *Diabetes* **2005**, *54*, 2988.,

(e)



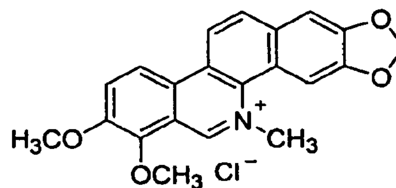
Lys[Z(NO_2)]-piperidide (**10**, X = CH_2CH_2)
15 Lankas et al., *Diabetes* **2005**, *54*, 2988.,

(f)



20 sanguinarine
Sedo et al., *Physiol. Res.* 2003, *52*, 367,

(g)

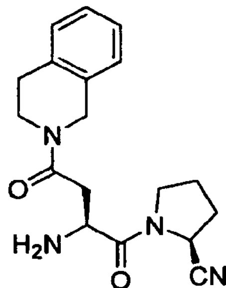


25

chelerythrine
Sedo et al., *Physiol. Res.* 2003, *52*, 367,

30

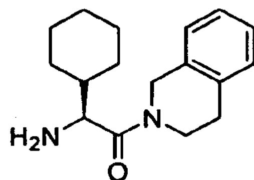
(h)



compound 5

5 Jiaang et al. Bioorg. Med. Chem. Lett. 15:687-691 (2005),

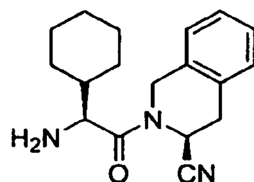
(i)



compound 7

10 Jiaang et al. Bioorg. Med. Chem. Lett. 15:687-691 (2005),

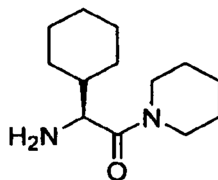
(j)



15 compound 8

Jiaang et al. Bioorg. Med. Chem. Lett. 15:687-691 (2005),

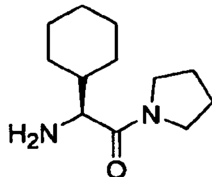
(k)



20 compound 13

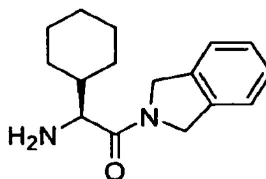
Jiaang et al. Bioorg. Med. Chem. Lett. 15:687-691 (2005),

(l)



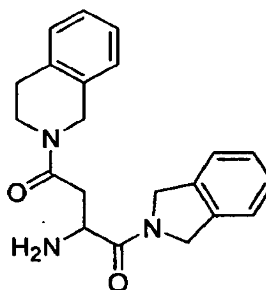
compound 14
5 Jiaang et al. Bioorg. Med. Chem. Lett. 15:687-691 (2005),

(m)



10 compound 15, PTX-1200
Jiaang et al. Bioorg. Med. Chem. Lett. 15:687-691 (2005),

(n)



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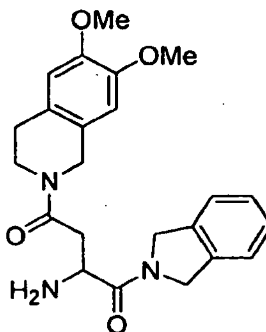
compound 16
Jiaang et al. Bioorg. Med. Chem. Lett. 15:687-691 (2005),

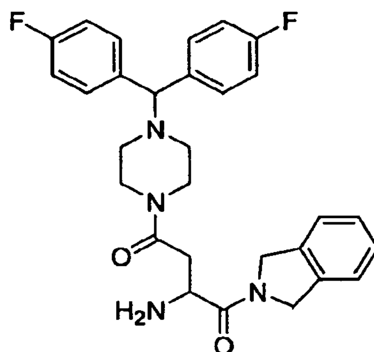
(o)



20

compound 17
Jiaang et al. Bioorg. Med. Chem. Lett. 15:687-691 (2005),

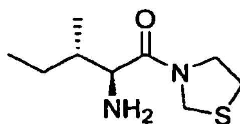
(p)



compound 18, PTX-1210

5 Jiaang et al. Bioorg. Med. Chem. Lett. 15:687-691 (2005),

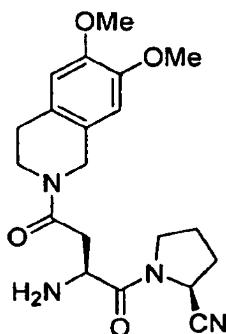
(q)



compound 4

10 Lu et al. Bioorg. Med. Chem. Lett. 15:3271-3275 (2005),

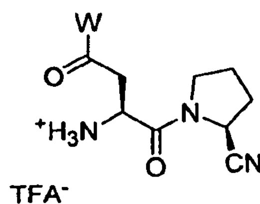
(r)



15 compound 8b

Lu et al. Bioorg. Med. Chem. Lett. 15:3271-3275 (2005),

(s)

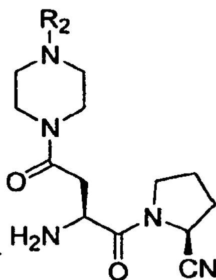


20

compound 9 W = piperazine derivatives

Lu et al. Bioorg. Med. Chem. Lett. 15:3271-3275 (2005),

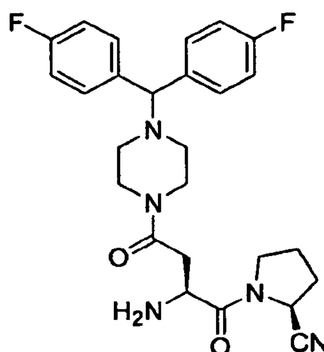
(t)



compound 9
Lu et al. Bioorg. Med. Chem. Lett. 15:3271-3275 (2005),

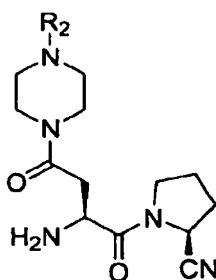
5

(u)



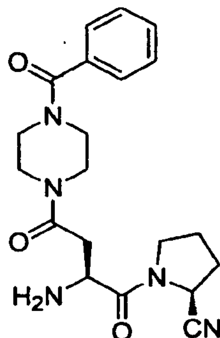
compound 9a
10 Lu et al. Bioorg. Med. Chem. Lett. 15:3271-3275 (2005),

(v)



15 wherein R_2 is nicotinonitrile.
compound 9b
Lu et al. Bioorg. Med. Chem. Lett. 15:3271-3275 (2005),

(w)



compound 9c (with R2 is benzoyl)
5 Lu et al. Bioorg. Med. Chem. Lett. 15:3271-3275 (2005),

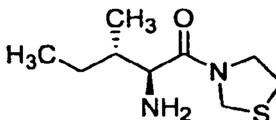
(x) Arg-Pro-Ala-Tyr (SEQ ID NO: 1 (WO02/31134),

(y) Met-Phe-Ala-Tyr (SEQ ID NO: 2 (WO02/31134) and

10 (z) Ile-Phe-Ala-Tyr (SEQ ID NO: 3 (WO02/31134).

Examples of DPP4 inhibitors including selective and specific DPP4 inhibitors are as follows:

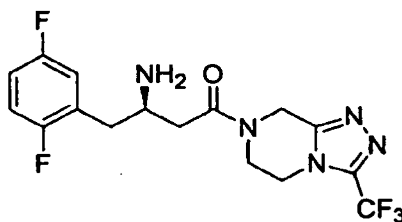
(1)



15

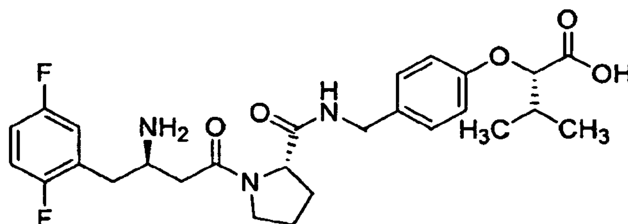
compound 1
L-*threo*-isoleucyl thiazolidide
Lankas et al., *Diabetes* **2005**, *54*, 2988.,

20 (2)



compound 3
Lankas et al., *Diabetes* **2005**, *54*, 2988.,

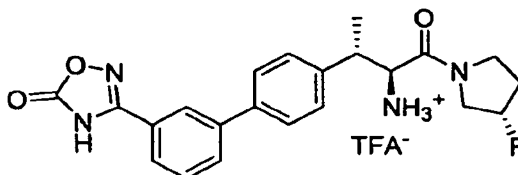
(3)



5 compound 6

Lankas et al., *Diabetes* **2005**, *54*, 2988.,

(4)

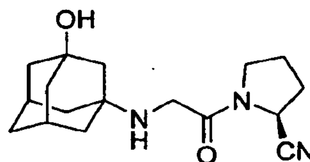


compound 44

10 5-oxo-1,2,4-oxadiazole

Xu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15(10)*, 2533.,

(5)



15

compound 1, LFA237

Vildagliptin ((2S-{[(3-hydroyxyadamantan-1-yl) amino]acetyl}-pyrrolidine-2-carbonitrile; Novartis)

I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

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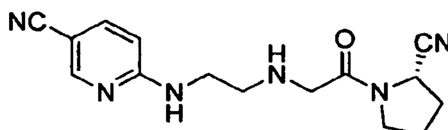
(6)

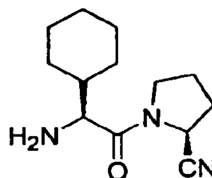


compound 2, DPP728

I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

25

(7)

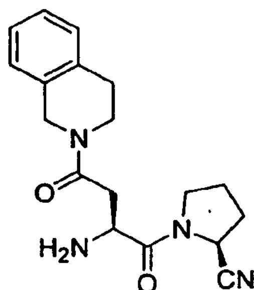


compound 3

I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

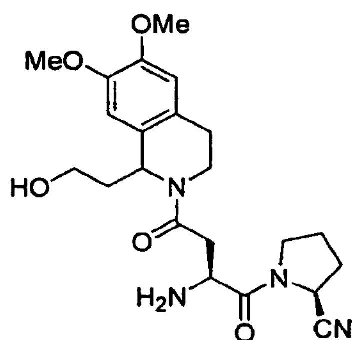
6

(8)



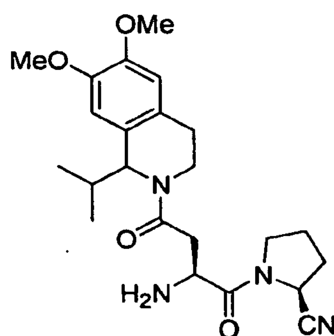
5 compound 8a
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

(9)



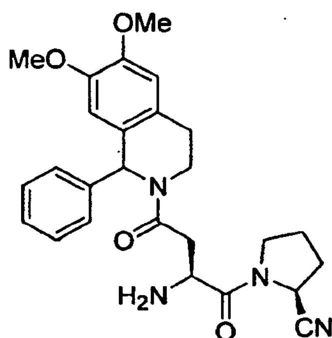
10 compound 8c
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

(10)



15 compound 8d
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

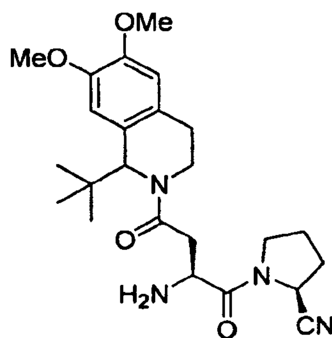
(11)



compound 8e
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

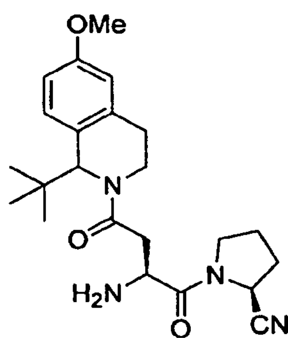
5

(12)



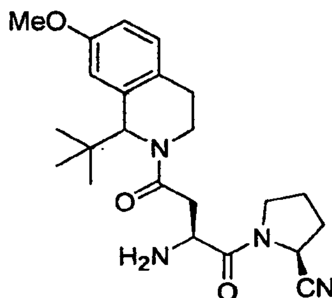
compound 8f
10 I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

(13)



15 compound 8g
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

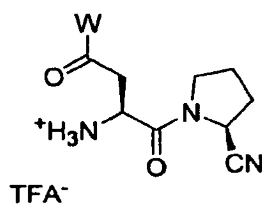
(14)



compound 8h
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

5

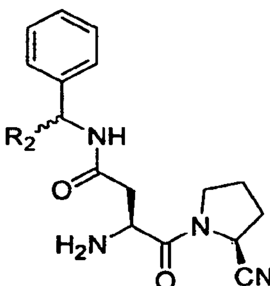
(15)



compound 10, W = benzyl derivatives
I-Lin Lu et al., *Bioorg. Med.. Chem. Lett.* **2005**, *15*, 3271.,

10

(16)



compound 10
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

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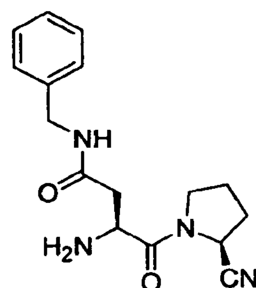
(17)



compound 10a
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

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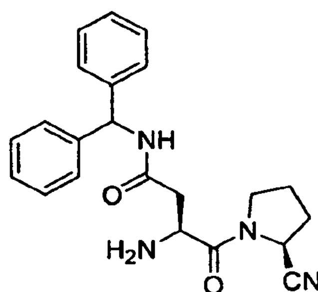

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I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

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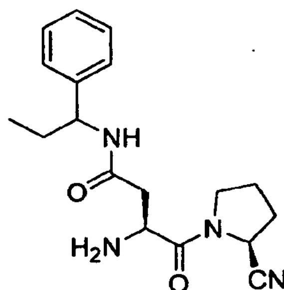

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I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

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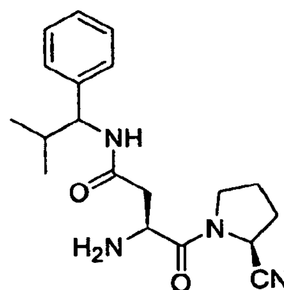

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compound 10d
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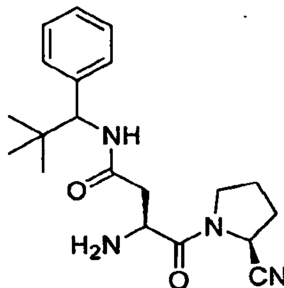

compound 10e
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

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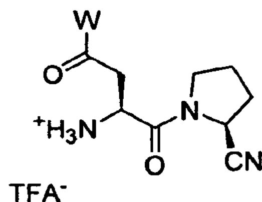

compound 11, W = phenethylamine derivatives
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

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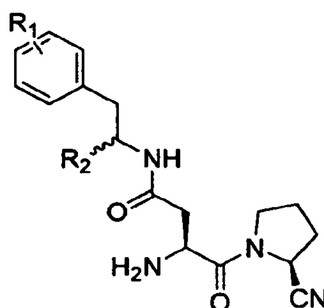

compound 11
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

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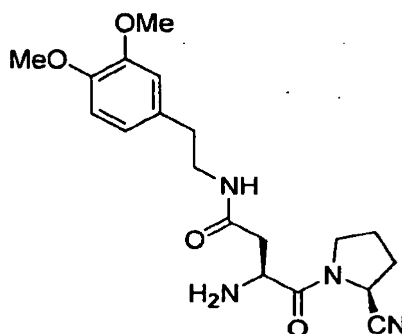

compound 11a
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.,

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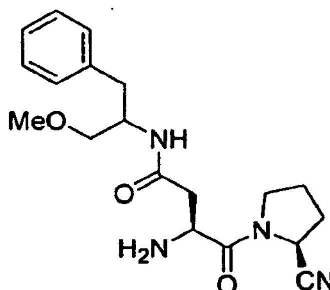

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I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271., and

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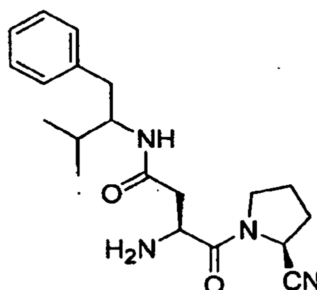
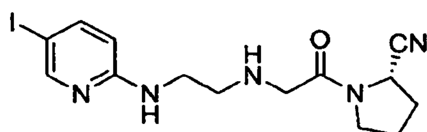


10 compound 11c
I-Lin Lu et al., *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271..

Still other DPP4 inhibitors are disclosed in Weber. J. Med. Chem. 2004, 47(17):4135
15 as follows:

1b



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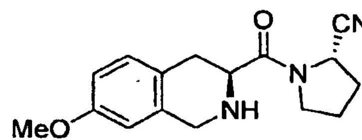

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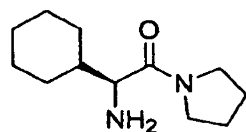

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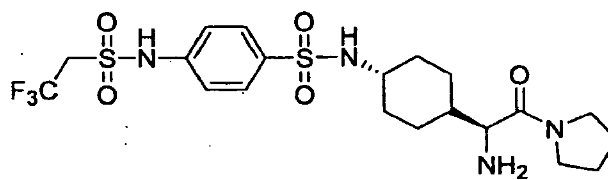

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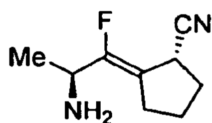

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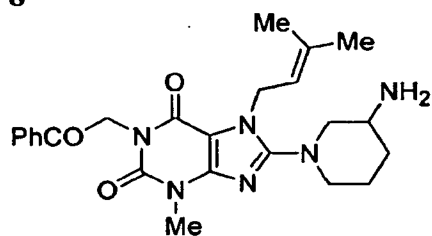

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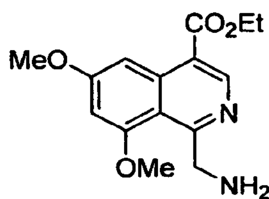

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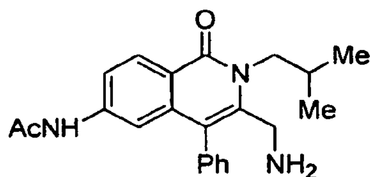

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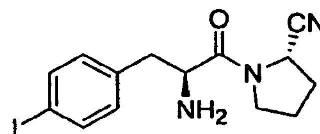

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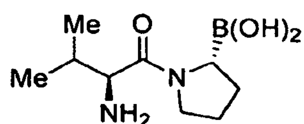
12a



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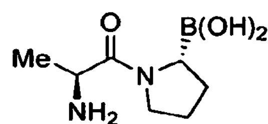

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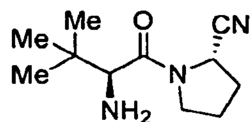
13a



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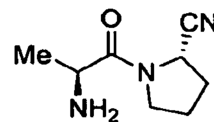

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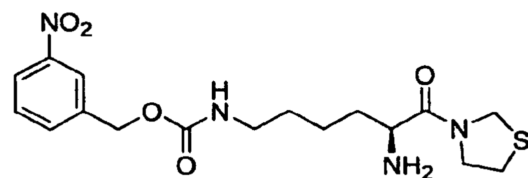

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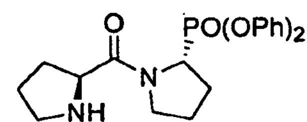

Other DPP4 inhibitors are Saxagliptin (BMS), Sitagliptin (Merck MK-0431), (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-α]pyrazin-7(8H)-yl]-1-(2,5-difluorophenyl)butan-2-amine fumarate and hydrochloride salts (des-fluoro analog of sitagliptin; Lankas et al. Diabetes 54:2988-2994, 2005, Merck), (2S)-2-[4-[[[(2S)-1-[(3R)-3-
5 amino-4-(2,5-difluorophenyl)-1-oxobutyl]-2-pyrrolidinyl]carbonyl]amino]methyl]phenoxy]-3-methylbutanoic acid, trifluoroacetate (Lankas et al. Diabetes 54:2988-2994, 2005, Merck), and various other compounds described in Lu et al. Bioorg Med Chem Lett 15:3271-3275, 2005; Jiaang et al. Bioorg Med Chem Lett 15:687-691, 2005; Xu et al. Bioorg Med Chem Lett 15:2533-2536, 2005; and Lankas et al. Diabetes 54:2988-2994, 2005. X-boroPro compounds
10 such as those described herein are also DPP4 inhibitors (e.g., Val-boroPro).

An example of a specific FAP inhibitor is acetyl-Gly-boroPro (Edosada et al. J Biol Chem. 2006 Mar 17;281(11):7437-44). Requirements for FAP specificity are discussed in Edosada et al. FEBS Lett. 2006 Mar 6;580(6):1581-6. X-boroPro compounds such as those described herein are also FAP inhibitors (e.g., Val-boroPro).

15 IC_{50} values for several of the afore-mentioned inhibitors are shown in Table A.

Table A: IC50 for DPP enzyme inhibitors

Compound/Source	DPP4 IC_{50}	FAP IC_{50}	DPP8 IC_{50}	DPP9 IC_{50}	PEP IC_{50}
DPP8/9 Inhibitors					
PTX-1200 (see Examples) compound 15, Jiaang et al.	>30000	>30000	25	60	>30000
PTX-1210 (see Examples) compound 18, Jiaang et al.	>30000	>30000	25	105	>30000
L-allo-isoleucyl thiazolidide (compound 2, Lankas et al.)	460	>100000	220	320	>100000
all-isoleucyl isoindoline (2S,3R)-2-(2-amino-3-methyl-1-oxopentan-1-yl)-1,3-dihydro-2H-isoindole hydrochloride (compound 4, Lankas et al.)	30000	>100000	38	55	>100000
Lys[Z(NO_2)]pyrrolidide (compound 8, Lankas et al.)	1300	51000	154	165	>100000
Lys[Z(NO_2)]thiazolidide (compound 9, Lankas et al.)	410	33000	210	75	56000
Lys[Z(NO_2)]piperidide (compound 10, Lankas et al.)	17000	>100000	1100	670	>100000
isoleucylthiazolidide, Ile-thia, P32/98 compound 4, Lu et al.	1660		364		
compound 8b, Lu et al.	63		19		

compound 9a, Lu et al. compound 6, Jiaang et al.	227-274		16		
compound 9b, Lu et al.	87		28		
compound 9c, Lu et al.	123		71		
compound 5, Jiaang et al.	63		19		
compound 7, Jiaang et al.	>100000		3016		
compound 8, Jiaang et al.	>100000		5962		
compound 13, Jiaang et al.	31000		1448		
compound 14, Jiaang et al.	1034		78		
compound 16, Jiaang et al.	>100000		555		
compound 17, Jiaang et al.	>100000		150		
quaternary benzo[c]pheanthridine alkaloid, QBA extract from Macleya cordata (EX), Sedo et al.					
DPP4 Inhibitors					
L-threo-isoleucyl thiazolidide (compound 1, Lankas et al.)	420	>100000	2180	1600	>100000
(2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-α]pyrazin-7(8H)-yl]-1-(2,5-difluorophenyl)butan-2-amine fumarate and hydrochloride salts (des-fluoro analog of sitagliptin) (compound 3, Lankas et al.)	27	>100000	69000	>100000	>100000
(2S)-2-[4-[[[[(2S)-1-[(3R)-3-amino-4-(2,5-difluorophenyl)-1-oxobutyl]-2-pyrrolidinyl]carbonyl]amino]methyl]phenoxy]-3-methylbutanoic acid, trifluoroacetate (compound 6, Lankas et al.)	0.48	21000	>100000	86000	39000
LFA237, Vildagliptin compound 1, Lu et al.	20	>25000	>14000	>14000	>200000
DPP728 compound 2, Lu et al.	53		4573		
cyclohexylglycine-(2S)-cyanopyrrolidine compound 3, Lu et al.	12		27		
compound 8a, Lu et al.	99		120		
compound 8c, Lu et al.	45		174		
compound 8d, Lu et al.	47		439		
compound 8e, Lu et al.	97		220		
compound 8f, Lu et al.	73		2196		
compound 8g, Lu et al.	72		1439		
compound 8h, Lu et al.	195		2712		
compound 10a, Lu et al.	238		389		

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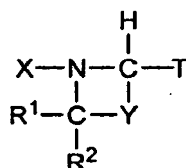
compound 10b, Lu et al.	252		2327		
compound 10c, Lu et al.	140		1412		
compound 10d, Lu et al.	212		3055		
compound 10e, Lu et al.	251		13217		
compound 11a, Lu et al.	116		177		
compound 11b, Lu et al.	182		2110		
compound 11c, Lu et al.	305		1597		
5-oxo-1,2,4-oxadiazole compound 44, Xu et al.					
PEP Inhibitors					
1-[2S-(benzylcarbmoylmethyl)-3-methylbutyryl]-pyrrolidine-2R-boronate	>50000	>25000	>50000	>50000	8
z-Pro-Pro-FMK	>50000	>50000	>50000	>50000	50
Val-Pro-boroPro	5000	15000	3000	3000	10
X-boroPro Compounds					
Val-boroPro	1	32	3	3	50

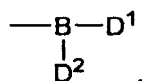
Table A lists a number of selective and specific inhibitors for various post-prolyl cleaving enzymes (i.e., comparison of IC50 values of a given compound for a number of post-prolyl cleaving enzymes will identify selective and specific inhibitors). Comparison between compounds is not intended as not all compounds are tested under identical conditions.

Further examples of inhibitors include isoleucylthiazolidide and cyclohexylglycylpyrrolidide.

Some methods employ inhibitors having the following structure

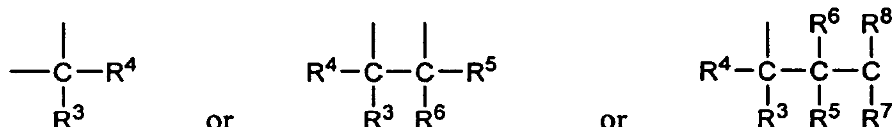


wherein T is a reactive group such as those recited above (e.g., a boronate group of the formula



each D^1 and D^2, independently, is a hydroxyl group or a group which is capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH); X comprises an amino acid or a peptide which mimics the site of a substrate recognized by a post-prolyl

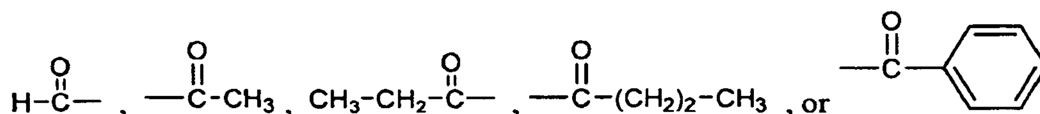
cleaving enzyme. In some embodiments, X further comprises an N-terminal blocking group. Y may be



5 Each R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8, independently, may be a group which does not interfere significantly with site-specific recognition of the compound by a post-prolyl cleaving enzyme.

In preferred embodiments, T is a boronate group, each D^1 and D^2, independently, is OH; each R^{1-8} is H; and X comprises an amino acid and a blocking group (e.g., N-blocking 10 group) such as an acetyl group.

The structure of blocking groups can vary widely. In one blocking reaction, a hydrogen atom of the amino terminal amino group is replaced, generally in a dehydration reaction. Blocking groups can be



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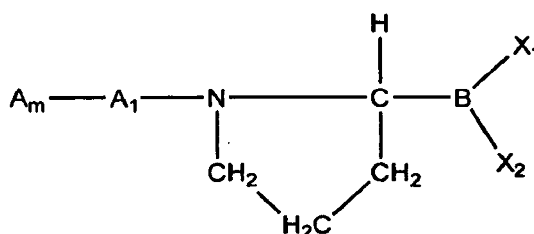
These blocking groups may be employed not only to protect amino-terminal groups (and thereby act as N-terminal blocking groups), but also may be used to protect side chains of amino acid residues (e.g., side chains of Lys and Arg). Similarly, amino acid residues having acidic or hydroxy side chains can be protected using t-butyl, benzyl, or other suitable 20 esters or ethers as blocking groups.

These and other inhibitors are described in US Patents 4935493, 5462928, 5965532, 6355614, 6825169, 6875737, and 6890904.

Other inhibitors have the following structure



wherein m is an integer between 0 and 10, inclusive; A and A_1 may be a naturally or non-naturally occurring amino acid residue, peptide or peptidomimetic such that when A is an amino acid residue each A in A_m (i.e., where m >1) may be a different amino acid residue from every other A in A_m and when A is a peptide or peptidomimetic m is 1; the C bonded to B is preferably in the R-configuration; and each X_1 and X_2 is, independently, a hydroxyl group or a group capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. A_1 may be valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, tryptophan, serine, threonine, aspartate, glutamate, asparagine, glutamine, lysine, arginine, histidine, cysteine, alanine or proline. Preferably, A_1 is an L- amino acid residue, and optionally every A in A_m is an L- amino acid residue.

In some embodiments m is 0. In some embodiments X_1 and X_2 are hydroxyl groups. The proline residue may be replaced with another amino acid residue such as, for example, alanine. As well, derivatives of these compounds in which the organo boronate is replaced with 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 are also contemplated by the invention. 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 where the compound comprises a dipeptide, the dipeptide moiety can be an isostere.

Still other inhibitors have the following structure

$$\left[A-N(CH_2CH_2CH_2)C(H)-C(=O) \right]_m - A_1 - N(CH_2CH_2CH_2)C(H)-B(X_1)(X_2)$$

wherein m is an integer between 0 and 10, inclusive; A and A_1 are naturally or non-naturally occurring amino acids, peptides or peptidomimetics; wherein if A is an amino acid residue, it can be a different amino acid residue in each repeating bracketed unit; the C bonded to B is in

the R-configuration; and each X_1 and X_2 is, independently, a hydroxyl group or a group capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH. A and A_1 may be alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, serine, threonine, aspartate, glutamate, asparagine, glutamine, lysine, arginine, histidine, cysteine, methionine, or proline. Preferably, A_1 is an L- amino acid residue, and optionally every A is an L- amino acid residue.

The amino acid residues may be naturally and non-naturally occurring amino acids. Examples of naturally occurring amino acids are glycine (Gly), and the L-forms of alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), 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 (Glu), asparagine (Asn), glutamine (Gln) and proline (Pro).

Non-naturally occurring amino acids include the D-forms of Ala, Val, Leu, Ile, Phe, Tyr, Trp, Cys, Met, Ser, Thr, Lys, Arg, His, Asp, Glu, Asn, Gln, 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 (Nle), 5-hydroxynorleucine (Hyn), 6-hydroxynorleucine, ornithine, cyclohexylglycine (Chg), N-Methylglycine (N-MeGly), N-Methylalanine (N-MeAla), N-Methylvaline (N-MeVal), N-Methylleucine (N-MeLeu), N-Methylisoleucine (N-MeIle), N-Methylnorleucine (N-MeNle), N-Methyl-2-aminobutyric acid (N-MeAbu) and N-Methyl-2-aminopentanoic acid (N-MeNva), methylthreonine, nitroglutamine, norleucine (Nle), norvaline, ornithine, phosphoserine, pipecolic acid, sarcosine, taurine, tert-leucine, thiazolidine carboxylic acid, thyroxine, trans-4-hydroxyproline, and trans-3-methylproline.

The boroPro inhibitors (and derivatives thereof including reactive group derivatives) 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 proportion of linear (versus cyclic) forms in these mixtures may vary (e.g., 0-100%) depending on the formulation. In some embodiment, at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of the inhibitor is in the linear form.

The boroPro inhibitors and derivatives may be provided as prodrugs that are converted (*via* enzymatic, chemical, metabolic, or any other means, *in vivo* or *ex vivo*) to the forms shown above. A prodrug of for example "A-boroPro", as used herein, is a compound that is metabolized *in vivo* to A-boroPro or that disintegrates (e.g., upon contact with stomach acid)

to form A-boroPro. Some prodrugs are converted into A-boroPro via hydrolysis or oxidation *in vivo*. These include alcohol precursors of A-boroPro that are oxidized *in vivo* (e.g., in the liver) and a boroxine derivative of A-boroPro, as well as esters of Glu-boroPro and related compounds. Prodrugs of A-boroPro also include cyclized versions of the molecule.

5 Another category of prodrugs includes compounds that are converted to A-boroPro by enzymes. These enzymes may be post-prolyl cleaving enzymes (e.g., DPP4) 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
10 published April 29, 1993; and WO 03/092605, published November 13, 2003, among others. The length of such prodrug compounds may be 4, 6, 8, 10, 12, 14, 16, 18, 20, 30, 50, 100 or more residues in length (whereby the length includes A and proline residues). Multiples of 3 are also contemplated.

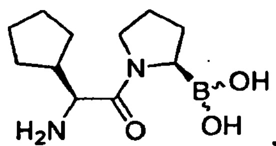
The boroPro inhibitors, when provided as a plurality or in a mixture, may be provided
15 in a substantially optically pure form. That is, at least 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% of these inhibitors in a mixture (or composition) possess boron-bearing carbon atoms that are in the L-configuration. Methods for synthesizing substantially optically pure isomers of boroPro inhibitors are disclosed in published PCT application WO 93/08259. In related embodiments, the mixture of R- and S-enantiomers of boron substituted pyrrolidine contains
20 at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the R-enantiomer of boron substituted pyrrolidine.

In some embodiments, the DPP8/9 inhibitors exclude the inhibitors described in U.S. Patent 6890904. The teachings of these inhibitors is incorporated by reference herein.

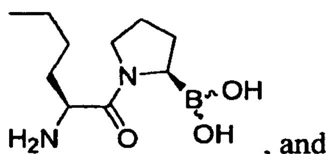
In some embodiments, the DPP8/9 inhibitor including a selective or specific DPP8/9
25 inhibitor is used together with Glu-boroPro, Gln-boroPro, Arg-boroPro, Phe-boroPro, Lys-boroPro, and/or acetyl-Gly-boroPro. The DPP8/9 inhibitors, including selective or specific DPP8/9 inhibitors and the X-boroPro compound may be used at their maximum tolerated dose. In these instances, the DPP8/9 inhibitor may be administered before, before and during, during and after, before and after, or before, during and after administration of the X-boroPro
30 compound.

In some embodiments, the DPP8/9 inhibitors, including selective or specific DPP8/9 inhibitors are used together with Val-boroPro, Ile-boroPro, Met-boroPro, Leu-boroPro, cyclopentylglycine boroPro

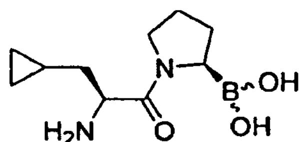
0



norleucine boroPro



5 cyclopropylalanine boroPro



The boroPro compound in these instances may be used at its maximum tolerated dose or at a sub-therapeutic dose. As used herein, a sub-therapeutic dose is a dose that is less than the 10 dose that produces the maximal, medically acceptable, therapeutic result in the subject when administered alone. The sub-therapeutic dose may be equal to or less than 95%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% or 5% of the therapeutic dose (i.e., the maximum tolerated dose), or it may be at least 50-fold, 100-fold, 500-fold, or 1000-fold less than that dose. Therapeutic doses for human subjects can be derived from clinical trial data. 15 Alternatively, therapeutic doses can be determined using murine model systems. In some instances, the sub-therapeutic dose of for example Val-boroPro may be a dose that reduces tumor size but does not induce cytokine or chemokine production.

Assays for determining effect of post-prolyl cleaving enzyme inhibitors on tumor growth in vivo are described in U.S. Pat. No. 6,890,904, issued on May 10, 2005, particularly 20 in Examples 2 and 3, and in U.S. Published Application 2005-0084490 A1, published on April 21, 2005, particularly in Examples 3 and 4, and such assay descriptions are incorporated by reference herein.

The invention further provides methods for identifying compounds as DPP8/9 inhibitors including selective or specific DPP8/9 inhibitors and using such compounds in the 25 methods provided herein. DPP8/9 inhibition assays include IC50 assays (as described herein) as well as cytokine and/or chemokine induction assays as described in the Examples as well

as in U.S. Published Application 2005-0084490 A1, published on April 21, 2005, particularly in Examples 1, 5, 6, and 7, and such assay descriptions are incorporated by reference herein.

Compounds that may be tested for DPP8/9 inhibition include but are not limited to those described in US Pat. No. 4,318,904; US Pat. No. 4,499,082; US Pat. No. 4,652,552; US Pat. No. 4,963,655; US Pat. No. 5,093,477; US Pat. No. 5,187,157; US Pat. No. 5,242,904; US Pat. No. 5,250,720; US Pat. No. 5,288,707; US Pat. No. 5,296,604; US Pat. No. 5,384,410; US Pat. No. 5,444,049; US Pat. No. 5,527,923; US Pat. No. 5,543,396; US Pat. No. 5,624,894; US Pat. No. 5,939,560; US Pat. No. 6,090,786; US Pat. No. 6,201,132; US Pat. No. 6,303,661; US Pat. No. 6,500,804; US Pat. No. 6,548,481; US Pat. No. 6,803,357; US Pat. No. 6,890,905; US Pat. No. 6,946,480; US Pat. App. No. 2002-0006899; US Pat. App. No. 2002-0198242; US Pat. App. No. 2003-0008905; US Pat. App. No. 2003-0119736; US Pat. App. No. 2003-0119750; US Pat. App. No. 2003-0130199; US Pat. App. No. 2003-0134802; US Pat. App. No. 2003-0135023; US Pat. App. No. 2003-0148961; US Pat. App. No. 2003-0153509; US Pat. App. No. 2003-0162820; US Pat. App. No. 2003-0176357; US Pat. App. No. 2003-0220267; US Pat. App. No. 2004-0167191; US Pat. App. No. 2004-0176307; US Pat. App. No. 2004-0229848; US Pat. App. No. 2005-0043299; US Pat. App. No. 2005-0171025; US Pat. App. No. 2005-0203027; US Pat. App. No. 2005-0209249; US Pat. App. No. 2005-0215603; US Pat. App. No. 2005-0215784; DD 158109; DD 294711; DD 296075; DE 1983 4591; EP 0 356 223; EP 0 371 467; EP 0 471 651; EP 0 481 311; EP 0 615 978; EP 0 688 788; EP 0 995 440; EP 1 084 705; WO 92/12140; WO 93/10127; WO 94/03055; WO 94/20526; WO 94/28915; WO 94/29335; WO 95/11689; WO 95/12618; WO 95/15309; WO 95/29190; WO 95/29691; WO 95/34538; WO 97/40832; WO 97/45117; WO 98/25644; WO 99/38501; WO 99/67278; WO 03/092605; WO 2005/106021; WO 2005/106487; AHN et al., Chem Pharm Bull, 2005, v53, p1048-50; BACHOVCHIN et al., J Biol Chem. 1990 Mar 5;265(7):3738-43.; BAKER et al., Biochemistry. 1983 Apr 26;22(9):2098-103.; BORLOO et al., Verh K Acad Geneeskd Belg. 1994;56(1):57-88.; COUTTS et al., Tetrahed Letts, 1994, v35, p5109-12.; DEMUTH et al., FEBS Lett. 1993 Mar 29;320(1):23-7.; FLENTKE et al., Proc Natl Acad Sci U S A. 1991 Feb 15;88(4):1556-9.; GIBSON et al., Org Proc and Dev, 2002, v6, p814-16.; GÜNTHER et al., Magn Reson Chem. 1995;33:959-70.; GUTHEIL et al., Biochemistry. 1993 Aug 31;32(34):8723-31.; HEGEN et al., Immunobiology. 1993 Dec;189(5):483-93.; HEINS et al., Biochim Biophys Acta. 1988 May 18;954(2):161-9.; JIANG et al., Res Virol. 1997 Jul-Aug;148(4):255-66.; KELLY et al., J Am Chem Soc. 1993;115:12637-8.; KELLY et al., Tetrahedron. 1993;49:1009-16.;

KETTNER et al., Biochemistry. 1988 Oct 4;27(20):7682-8.; KETTNER et al., Chemical Abstracts. 1990;112:80. Abstract number 91790c.; KIEFFER eta l., Endocrinology, 1995, v136, p3585.; KINDER et al., Invasion Metastasis. 1992;12(5-6):309-19.; KINDER et al., J Med Chem. 1985 Dec;28(12):1917-25.; KINDER et al., J Med Chem. 1990 Feb;33(2):819-23.; KUBOTA et al., Clin Exp Immunol. 1992 Aug;89(2):192-7.; MATTESON et al., Organometallics. 1984;3(8):1284-8.; MENTLEIN et al., 1993, v214, p829.; MORIMOTO et al., J Immunol. 1989 Dec 1;143(11):3430-9.; REINHOLD et al., Immunol Lett. 1997 Jun;58(1):29-35.; SCHON et al., Biol Chem Hoppe Seyler. 1991 May;372(5):305-11.; SCHON et al., Biomed Biochim Acta. 1985;44(2):K9-15.; SCHON et al., Biomed Biochim Acta. 1986;45(11-12):1523-8.; SCHON et al., Eur J Immunol. 1987 Dec;17(12):1821-6.; SNOW et al., J Am Chem Soc. 1994;116:10860-9.; SUDMEIER et al., Biochemistry. 1994 Oct 18;33(41):12427-38.; THOMPSON et al., Biochemistry. 1973 Jan 2;12(1):47-51.; THOMPSON et al., in Methods in Enyzmology. Colowick et al., eds. Chap 19, 46: 220-5.; UNDERWOOD et al., J Bio Chem 1999, v274, p34053.; WELCH et al., Tetrahedron. 1996 January 1;52(1):291-304.; WOOD et al., J Med Chem. 1989 Oct;32(10):2407-11.; YOSHIMOTO et al., J Biochem (Tokyo). 1985 Oct;98(4):975-9.

The invention further provides methods for screening compounds for DPP8/9 inhibition followed by ability to stimulate immune responses as described herein. For example, the compounds may be screened for the ability to induce IL-1 (e.g., IL-1beta) levels as described in the Examples. Alternatively, or additionally, the compounds may be screened for the ability to induce levels of other cytokines and/or chemokines (e.g., IL-6). These assays may be conducted in vitro or in vivo.

Further aspects of the invention are based in part on the discovery that DPP8/9 inhibitors, and particularly selective or specific DPP8/9 inhibitors, act at least in part via neutrophil and macrophage activation and recruitment. Based in part on this finding, the invention provides methods of treating conditions amenable to immunotherapy (e.g., cancer or infectious disease) by inducing neutrophil and/or macrophage production and/or chemotaxis to affected sites in the body. Neutrophil production can be induced using granulocyte stimulating factors. Granulocyte stimulating factors are factors that increase the number of granulocytes, and preferably neutrophils, in the subject. Increased granulocyte numbers generally result from increased differentiation from precursors including granulocyte restricted precursors such as CFC-G or oligo- or multi-lineage precursors such as CFC-GM or CFC-GEMM. Granulocyte stimulating factors are known in the art and include but are not

limited to granulocyte colony stimulating factor (G-CSF) and granulocyte/macrophage colony stimulating factor (GM-CSF). Similarly, macrophage production can be induced using macrophage stimulating factors. Macrophage stimulating factors are factors that increase the number of macrophages in the subject. Increased macrophage numbers generally result from increased differentiation from precursors including macrophage restricted precursors such as CFC-M or oligo- or multi-lineage precursors such as CFC-GM or CFC-GEMM. An example of a macrophage stimulating factor is macrophage colony stimulating factor (M-CSF) which is known in the art. In some embodiments, the invention contemplates the administration of a granulocyte and/or a macrophage stimulating factor prior to the administration of a DPP8/9 inhibitor including a selective or specific DPP8/9 inhibitor.

Other aspects and embodiments of the invention also provide for the use of chemoattractants for particular immune cell types including neutrophils and T cells. Chemoattractants are agents that induce movement of cells along a concentration gradient (e.g., from a region of low chemoattractant concentration to a region of higher chemoattractant concentration). Examples of chemoattractants are provided in Table B. In some embodiments, the chemoattractants are administered locally to an affected site (e.g., a melanoma lesion). The chemoattractants may also be administered locally in a sustained release formulation.

Table B. Chemoattractants

Receptor	Target cell type	Chemokine
CXCR1 and CXCR2	Neutrophil	CXCL8/IL-8, CXCL6/GCP-2, CXCL1/Gro-α, CXCL1/Gro-β/MIP-2α, CXCL3/Gro-γ, CXCL7/NAP-2, CXCL5/ENA 78
CXCR3	T cell, vascular endothelial cells (anti-angiogenic)	CXCL10/IP10, CXCL9/MIG
CXCR4	Neutrophil, monocyte, T cell	CXCL12/SDF-1α
CCR1	Monocyte, eosinophils, basophil, T cell	CCL5/RANTES, CCL3L1/MIP-1α/LD78β, CCL8/MCP-2, CCL7/MCP-3
CCR2	Monocyte, basophil, T cell	CCL2/MCP-1, CCL8/MCP-2, CCL7/MCP-3, CCL13/MCP-4
CCR3	Eosinophil, basophil, T cell	CCL11/eotaxin, eotaxin-2, CCL5/RANTES, CCL8/MCP-2, CCL7/MCP-3, CCL13/MCP-4
CCR4	T cell	TARC
CCR5	Monocytes	CCL5/RANTES, CCL3L1/MIP-1α/LD78β, CCL4/MIP-1β

4

CCR6	T cell	LARC/MIP-3a/exodus
CCR7	T cell	ELC/MIP-3β

The invention intends to treat subjects having or at risk of developing a condition characterized by abnormal mammalian cell proliferation. As used herein, subject means a mammal including humans, nonhuman primates, dogs, cats, sheep, goats, horses, cows, pigs and rodents. As used herein, an abnormal mammalian cell proliferation disorder or condition refers to a cell population (e.g., a tumor) which exhibits an abnormal (e.g., increased) rate of division as compared to its normal cellular counterparts, or which grows in a factor independent manner. Conditions characterized by an abnormal mammalian cell proliferation, as used herein, include but are not limited to solid and non-solid benign, pre-malignant or malignant conditions.

The condition may be a cancer. The cancer may be carcinoma, sarcoma or melanoma. 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.

Carcinomas include but are not limited to basal cell carcinoma, biliary tract cancer, bladder cancer, breast cancer, cervical cancer, choriocarcinoma, CNS cancer, colon and rectum cancer, kidney or renal cell cancer, larynx cancer, liver cancer, small cell lung cancer, non-small cell lung cancer (NSCLC, including adenocarcinoma, giant (or oat) cell carcinoma, and squamous cell carcinoma), oral cavity cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer (including basal cell cancer and squamous cell cancer), stomach cancer, testicular cancer, thyroid cancer, uterine cancer, rectal cancer, cancer of the respiratory system, and cancer of the urinary system.

Sarcomas are rare mesenchymal neoplasms that arise in bone (osteosarcomas) and soft tissues (fibrosarcomas). Sarcomas include liposarcomas (including myxoid liposarcomas and pleiomorphic 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., not bone)

Ewing's sarcoma, and primitive neuroectodermal tumor), synovial sarcoma, angiosarcomas, hemangiosarcomas, lymphangiosarcomas, Kaposi's sarcoma, hemangioendothelioma, 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), and chondrosarcoma.

Melanomas are tumors arising from the melanocytic system of the skin and other organs. Examples of melanoma include lentigo maligna melanoma, superficial spreading melanoma, nodular melanoma, and acral lentiginous melanoma.

The cancer may be a leukemia or a lymphoma. Examples include acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), chronic myeloid leukemia (CML), chronic lymphoid (or lymphocytic) leukemia (CLL), T cell leukemia, B cell leukemia, Hodgkin's lymphoma, Non-Hodgkin's lymphoma, B cell lymphoma, and myeloma.

The cancer may be bone cancer, brain cancer, connective tissue cancer, cancer of the digestive system, endometrial cancer, esophageal cancer, eye cancer, cancer of the head and neck, gastric cancer, intra-epithelial neoplasm, neuroblastoma, retinoblastoma, rhabdomyosarcoma.

In important embodiments, the cancer is breast cancer, colorectal cancer, chronic lymphocytic leukemia, non-small cell lung cancer, Non-Hodgkin's lymphoma, melanoma or prostate cancer.

The condition may be a primary tumor with undetectable metastatic lesions, or it may be a tumor that has metastasized. The compositions and methods provided herein therefore are intended to treat primary tumors and/or metastases. In some instances, treatment may be of a primary tumor in the absence of detectable metastatic lesions in the subject.

The cancer may or may not express DPP8/9. Some cancers to be treated according to the invention are DPP8/9 negative (as measured by mRNA and/or protein expression assays). Others may be DPP8/9 positive but with an expression level that is regarded as normal when compared to the expression level of DPP8/9 in normal tissue counterparts.

In still other embodiments, the invention intends to treat hormone-insensitive cancers. Hormone insensitive cancers are cancers that do not require hormones for survival. Examples of these cancers include breast cancer, prostate cancer, testicular cancer, and some forms of ovarian cancer. Examples of hormones include estrogen, progesterone, and testosterone.

In some embodiments, the anti-cancer effect of DPP8/9 inhibitors is a T cell independent effect (i.e., it occurs independently of T cell involvement). In some instances, such a T cell independent effect can be observed in osteosarcoma, non-small cell lung carcinoma, head and neck cancer, and pancreatic cancer. In these and other cancer types, the anti-cancer effect of DPP8/9 inhibitors appears may involve innate immune responses including those involving neutrophils.

In some embodiments, the anti-cancer effect of DPP8/9 inhibitors involves T cells including CTLs. These effects can be observed in fibrosarcoma, T cell lymphoma and mastocytoma.

In still other embodiments, both T cells and neutrophils are believed to be involved in the anti-cancer effects of DPP8/9 inhibitors. These effects can be observed in B cell lymphoma and melanoma.

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. Refractory 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, or surgery, or radiation, or a combination thereof. Those of ordinary skill in the art are aware of such standards of care or are able to readily discern the ordinary standard of care based on FDA guidelines. 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, melanoma, lung cancer including non-small cell lung cancer, pancreatic cancer and Non-Hodgkin's lymphoma. The ordinary standard of care may comprise one or more chemotherapeutic or immunotherapeutic agents including but not limited to platinum-containing regimens such as cisplatin and carboplatin, docetaxel (TAXOTERE), pemetrexed (ALIMTA), 5-fluorouracil, gemcitabine, cyclophosphamide, doxorubicin, rituximab (RITUXAN), HERCEPTIN, and the like, either alone or in combination, concurrently or consecutively administered during treatment of the subject. Accordingly, the methods provided herein may be used as first line therapy or as subsequent line therapy (e.g., second, third or fourth line therapy).

The invention can also 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. These cancers therefore are also known to respond to immunotherapy such as vaccine or antibody therapy. Examples of such cancers include melanoma, renal cell cancer, colorectal cancer, breast cancer, ovarian cancer, prostate cancer, leukemia such as T cell leukemia, B cell leukemia, acute myelogenous leukemia, chronic myelogenous leukemia, common (pre-B) acute lymphocytic leukemia, chronic lymphocytic leukemia (CLL), and lymphoma such as Non-Hodgkin's lymphoma, T-cell lymphoma, and B-cell lymphoma.

One category of benign or precancerous conditions characterized by abnormal cell proliferation is proliferative dermatologic disorders. These include conditions such as keloids, actinic keratosis, bowenoid actinic keratosis seborrheic keratosis, hemangiomas, papilloma virus infection (e.g. producing verruca vulbaris, verruca plantaris, verruca plana, condylomata, etc.), eczema, hypertrophic actinic keratosis, arsenical keratosis, hydrocarbon keratosis, thermal keratosis, radiation keratosis, viral keratosis, Bowen's disease, erythroplaquia of queyrat, oral erythroplaquia, leukoplakia, and intraepidermal epithelialoma. An precancerous lesion is a lesion that has a propensity to develop into a cancerous condition. In some cases, the epithelial lesions may develop into an invasive form of squamous cell carcinoma and may pose a significant threat of metastasis.

The compositions and methods of the invention are useful in some instances for improving the efficacy of or replacing existing therapies (e.g., cancer therapies) including but not limited to surgical procedures, radiation therapies, chemotherapies, immunotherapies and/or hormone therapies (e.g., the ordinary standard of care therapies). Accordingly the DPP8/9 inhibitors including selective or specific DPP8/9 inhibitors can be used in combination with surgery, radiation, chemotherapy, immunotherapy and/or hormone therapy to treat subjects according to the invention. The second therapy (i.e., the non-DPP8/9 inhibitor therapy) may be administered before, concurrent with, or after treatment with DPP8/9 inhibitor. 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 inhibitor(s) may be administered before or after the other treatment.

Thus, the DPP8/9 inhibitors including the selective or specific DPP8/9 inhibitors may be administered, according to some embodiments, with a second therapeutic agent. The

second therapeutic agent may be an anti-cancer agent or an anti-microbial agent but it is not so limited. The second therapeutic agent may be but is not limited to a chemotherapeutic agent or an immunotherapeutic agent. The immunotherapeutic agent may be but it not limited to an antigen (e.g., in the form of a vaccine), an antibody or an antibody fragment, an adjuvant, a cytokine, or a chemokine.

An anti-cancer agent is an agent that at least partially inhibits the development or progression of a cancer, including inhibiting in whole or in part symptoms associated with the cancer even if only for the short term. The anti-cancer agent may be a chemotherapeutic agent. The compositions of the invention can further include chemotherapeutic agents such as but not limited to those currently in use with the antibodies recited herein. Several chemotherapeutic 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), DNA strand break inducing agents (e.g., bleomycin, doxorubicin, daunorubicin, idarubicin, mitomycin C), and radiation therapy.

In some embodiments, the DPP8/9 inhibitor is used alone or in conjunction with a DPP4 inhibitor and/or a FAP inhibitor and optionally with cisplatin in the treatment of melanoma (e.g., metastatic melanoma). Any of all of these inhibitors may be selective or specific inhibitors, although they are not so limited. In some embodiments, the DPP8/9 inhibitor is used alone or in conjunction with a DPP4 inhibitor and/or a FAP inhibitor and optionally with docetaxel (TAXOTERE) and/or pemetrexed (ALIMTA) in the treatment of non-small cell lung carcinoma. In some embodiments, the DPP8/9 inhibitor is used alone or in conjunction with a DPP4 inhibitor and/or a FAP inhibitor and optionally with rituximab (RITUXAN) in the treatment of chronic lymphocytic leukemia. In some embodiments, the DPP8/9 inhibitor is used alone or in conjunction with a DPP4 inhibitor and/or a FAP inhibitor and optionally with gemcitabine in the treatment of pancreatic cancer.

Some important chemotherapies include but are not limited to docetaxel (TAXOTERE) (e.g., in non-small cell lung carcinoma), pemetrexed (ALIMTA), gemcitabine, cisplatin (e.g., in lymphoma and melanoma), carboplatin, cyclophosphamide, doxorubicin,

dacarbazine, 5-FU, paclitaxel (TAXOL), erlotinib (TARCEVA), gefitinib (IRESSA), temozolomide (TEMODAR), imatinib mesylate (GLEEVAC), lenalidomide (REVLIMID, REVIMID), temozolomide (TEMODAR), thalidomide (THALOMID), bortezomib (VELCADE), rituximab (RITUXAN) (e.g., in lymphoma or CLL), trastuzumab
5 (HERCEPTIN), and bevacizumab (AVASTIN) (e.g., in non-small cell lung carcinoma).

Further examples 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
10 Hydrochloride; Bisnafide Dimesylate; Bizelesin; Bleomycin Sulfate; Bortezomib (VELCADE); 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; Decitabine;
15 Dexormaplatin; Dezaguanine; Diaziquone; Docetaxel (TAXOTERE); Doxorubicin; Droloxifene; Dromostanolone; Duazomycin; Edatrexate; Eflornithine; Elsamitrucin; Enloplatin; Enpromate; Epipropidine; Epirubicin; Erbulozole; Erlotinib (TARCEVA), Esorubicin; Estramustine; Etanidazole; Etoposide; Etoprine; Fadrozole; Fazarabine; Fenretinide; Floxuridine; Fludarabine; Fluorouracil; Flurocitabine; Fosquidone; Fostriecin;
20 Gefitinib (IRESSA), Gemcitabine; Hydroxyurea; Idarubicin; Ifosfamide; Ilmofosine; Imatinib mesylate (GLEEVAC); Interferon alpha-2a; Interferon alpha-2b; Interferon alpha-n1; Interferon alpha-n3; Interferon beta-I a; Interferon gamma-I b; Iproplatin; Irinotecan; Lanreotide; Lenalidomide (REVLIMID, REVIMID); Letrozole; Leuprolide; Liarozole; Lometrexol; Lomustine; Losoxantrone; Masoprocol; Maytansine; Mechlorethamine;
25 Megestrol; Melengestrol; Melphalan; Menogaril; Mercaptopurine; Methotrexate; Metoprine; Meturedepa; Mitindomide; Mitocarcin; Mitocromin; Mitogillin; Mitomalcin; Mitomycin; Mitosper; Mitotane; Mitoxantrone; Mycophenolic Acid; Nocodazole; Nogalamycin; Ormaplatin; Oxisuran; Paclitaxel; Pemetrexed (ALIMTA), Pegaspargase; Peliomycin; Pentamustine; Pentomone; Peplomycin; Perfosfamide; Pipobroman; Piposulfan; Piritrexim
30 Isethionate; Piroxantrone; Plicamycin; Plomestane; Porfimer; Porfiromycin; Prednimustine; Procarbazine; Puromycin; Pyrazofurin; Riboprine; Rogletimide; Safingol; Semustine; Simtrazene; Sitogluside; Sparfosate; Sparsomycin; Spirogermanium; Spiromustine; Spiroplatin; Streptonigrin; Streptozocin; Sulofenur; Talisomycin; Tamsulosin; Taxol;

Taxotere; Tecogalan; Tegafur; Teloxantrone; Temoporfin; Temozolomide (TEMODAR); Teniposide; Teroxirone; Testolactone; Thalidomide (THALOMID) and derivatives thereof; Thiamiprine; Thioguanine; Thiotepa; Tiazofurin; Tirapazamine; Topotecan; Toremifene; Trestolone; Triciribine; Trimetrexate; Triptorelin; Tubulozole; Uracil Mustard; Uredepa;
5 Vapreotide; Verteporfin; Vinblastine; Vincristine; Vindesine; Vinepidine; Vinglycinate; Vinleurosine; Vinorelbine; Vinrosidine; Vinzolidine; Vorozole; Zeniplatin; Zinostatin; Zorubicin. When combined with a DPP8/9 inhibitor and optionally other inhibitors or therapeutic agents, the chemotherapeutic agent and/or any of the other active agents may be administered in a sub-therapeutic dose, as defined herein.

10 In some particular embodiments, the DPP8/9 inhibitor is used in combination with IL-1alpha. In these and other embodiments, one or more of the following chemotherapies can be administered to the subject in order to achieve a potentiated effect: doxorubicin, cisplatin, indomethacin, ketoconazole, mitomycin-D, porfiromycin, cyclophosphamide, and carboplatin.

15 The anti-cancer agent may be an immunotherapeutic agent. The immunotherapeutic agent may be an antibody or an antibody fragment. Examples include antibodies such as but not limited to bevacizumab (AVASTIN), trastuzumab (HERCEPTIN), alemtuzumab (CAMPATH, indicated for B cell chronic lymphocytic leukemia,), gemtuzumab (MYLOTARG, hP67.6, anti-CD33, indicated for leukemia such as acute myeloid leukemia),
20 rituximab (RITUXAN), tositumomab (BEXXAR, anti-CD20, indicated for B cell malignancy), MDX-210 (bispecific antibody that binds simultaneously to HER-2/neu oncogene protein product and type I Fc receptors for immunoglobulin G (IgG) (Fc gamma RI)), oregovomab (OVAREX, indicated for ovarian cancer), edrecolomab (PANOREX), daclizumab (ZENAPAX), palivizumab (SYNAGIS, indicated for respiratory conditions such
25 as RSV infection), ibritumomab tiuxetan (ZEVALIN, indicated for Non-Hodgkin's lymphoma), cetuximab (ERBITUX), MDX-447, MDX-22, MDX-220 (anti-TAG-72), IOR-C5, IOR-T6 (anti-CD1), IOR EGF/R3, celogovab (ONCOSCINT OV103), epratuzumab (LYMPHOCIDE), pemtumomab (THERAGYN), and Gliomab-H (indicated for brain cancer, melanoma). When combined with a DPP8/9 inhibitor and optionally other inhibitors or
30 therapeutic agents, the immunotherapeutic agent and/or any of the other active agents may be administered in a sub-therapeutic dose, as defined herein.

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, CD1, CEA, CD22, GD2 ganglioside, FLK2/FLT3, VEGFR, and the like.

The antibody or antibody fragment may be specific for CD20. Examples of such antibodies include rituximab (RITUXAN), ibritumomab tiuxetan (ZEVALIN), 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 may be specific for CD33. Examples include gemtuzumab (MYLOTARG, CMA 676) and SMART M195 (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 1D10 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 CD1. Examples include IOR-T6.

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-1, VCAM-1, ICAM-1, ligand reactive with LAM-1, MHC class II antigens, aminophospholipids such as phosphatidylserine and phosphatidylethanolamine (as described in U.S. Pat. No. 6,312,694), VEGFR1 (Flt-1) and

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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-11, and antibodies that recognize identical epitopes to these antibodies. Antibodies to aminophospholipids are described in U.S. Pat. No. 6,312,694.
5 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.

10 The antibody or antibody fragment may be specific for a stromal cell molecule such as but not limited to FAP and CD26.

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.

The antibody or antibody fragment may be specific for CA 125. Examples include
15 oregovomab (OVAREX, B43.13). The antibody or antibody fragment may be specific for MUC-1. Examples include BREVAREX. The antibody or antibody fragment may be specific for PSA. Examples include PROSTAREX. The antibody or antibody fragment may be specific for CA19.9. Examples include GIVAREX. The antibody or antibody fragment may be specific for Ep-CAM (EPG40 antigen, 17-1A). Examples include celogovab
20 (ONCOSCINT, OV103) and 3622W94. The antibody or antibody fragment may be specific for VEGF. Examples include bevacizumab (AVASTIN). Examples include MDX-220. The antibody or antibody fragment may also be an anti-idiotypic antibody. Examples include anti-idiotypic mAb mimic of high molecular weight proteoglycan (I-Mel-1), MELIMMUNE-1, melanoma (IDEC); anti-idiotypic mAb mimic of high molecular weight proteoglycan (I-Mel-
25 2), MELIMMUNE-2, melanoma (IDEC); and anti-idiotypic mAb mimic of ganglioside GD3 epitope, BEC2, small cell lung carcinoma, melanoma (ImClone Systems).

The antibody or antibody fragment may be infliximab (inflammatory bowel disease and rheumatoid arthritis) or etanercept (rheumatoid arthritis).

The antibody or antibody fragment may be conjugated (covalently or otherwise) to a
30 toxin derived from plant, fungus, or bacteria. 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 and Pseudomonas exotoxin, but is not so limited. The antibody or antibody fragment may also be conjugated to a

chemotherapeutic agent, a radioisotope or a cytotoxin. The chemotherapeutic agent 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.

The antibody or antibody fragment may be administered on a first day of multi-day cycle, with the DPP8/9 inhibitor (e.g., the selective or specific DPP8/9 inhibitor) administered on the remaining days of the cycle. The cycle may be a 2, 3, 4, 5, 6, 7, or more day cycle. The DPP8/9 inhibitor may be administered once, twice, or more times per day. In one embodiment, the antibody or antibody fragment is administered on the first day of a seven day cycle, followed by a twice daily administration of a DPP8/9 inhibitor on each of the remaining days of the seven day cycle. Other therapeutic agents may also be administered during the cycle. For example, the DPP4 and/or the FAP inhibitor may be administered on the same days as the DPP8/9 inhibitor, on alternating days (e.g., days 2, 4 and 6 of a seven day cycle), and the like. The multi-day cycle may be repeated twice, thrice, four times, or more. It may also be repeated for various lengths of time, including but not limited to a week, a month, two months, or more.

The DPP8/9 inhibitors may be used to stimulate immune responses to a cancer antigen. This may be accomplished using the inhibitors together with the cancer antigen itself or an antibody (or antibody fragment) specific to that antigen.

Cancer antigens are antigens that are expressed in cancerous cells at levels that are higher than the expression level in normal counterpart cells. The cancer antigen may be peptide, lipid or carbohydrate in nature or some combination thereof. 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.

Cancer antigens can be classified in a variety of ways. For example, cancer antigens include antigens that are encoded by mutant cellular genes such as oncogenes (e.g., activated ras oncogene) or suppressor genes (e.g., mutant p53), antigens that are fusion proteins resulting from internal deletions or chromosomal translocations; and antigens encoded by viral genes such as those carried on RNA and DNA tumor viruses.

Cancer antigens include antigens encoded by genes that have undergone chromosomal alteration. Many of these antigens are found in lymphoma and leukemia. Even within this classification, antigens can be characterized as those that involve activation of quiescent genes. These include *BCL-1 and IgH* (Mantel cell lymphoma), *BCL-2 and IgH* (Follicular lymphoma), *BCL-6* (Diffuse large B-cell lymphoma), *TAL-1 and TCR□ or SIL* (T-cell acute lymphoblastic leukemia), *c-MYC and IgH or IgL* (Burkitt lymphoma), *MUN/IRF4 and IgH* (Myeloma), *PAX-5 (BSAP)* (Immunocytoma).

Other cancer antigens that involve chromosomal alteration and thereby create a novel fusion gene and/or protein include *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), and *NPM, MLF-1* (Myelodysplastic syndrome/acute myeloid leukemia).

Other cancer antigens are specific to a tissue or cell lineage. These include cell surface proteins such as 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), and Prostate specific membrane antigen (Prostate cancer).

Tissue- or lineage- specific cancer antigens also include epidermal growth factor receptors (high expression) such as 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), and erbB4 (HER4) (Breast cancer). Tissue- or lineage- specific cancer antigens also include cell-associated proteins such as Tyrosinase, Melan-A/MART-1, tyrosinase related protein (TRP)-1/gp75 (Malignant melanoma), Polymorphic epithelial mucin (PEM) (Breast tumors), and Human epithelial mucin (MUC1) (Breast, ovarian, colon and lung cancers). Tissue- or lineage- specific cancer antigens also include secreted proteins such as 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), and Prostate specific antigen (Prostate cancer).

Still other cancer antigens are cancer testis (CT) antigens that 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 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. Still other examples of CT antigens and the cancers in which they are expressed include 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, PRAME, SSX-1 and CT-7 (Non-Hodgkin's lymphoma), and PRAME (Acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML) and chronic lymphocytic leukemia (CLL)).

Other cancer antigens are not specific to a particular tissue or cell lineage. These include members of the 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.

Still other cancer antigens are viral proteins and these include Human papilloma virus protein (cervical cancer), and EBV-encoded nuclear antigen (EBNA)-1 (lymphomas of the neck and oral cancer).

Still other cancer antigens are mutated or aberrantly expressed molecules such as but not limited to CDK4 and beta-catenin (melanoma).

These antigens include HER 2 (p185), 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 MOv18, high Mr melanoma antigens recognized by antibody 9.2.27, HMFG-2, SM-3, B72.3, PR5C5, PR4D2, and the like.

Other cancer antigens include MART-1/Melan-A, gp100, adenosine deaminase-binding protein (ADAbp), FAP, cyclophilin b, colorectal associated antigen (CRC)--C017-1A/GA733, carcinoembryonic antigen (CEA), CAP-1, CAP-2, etv6, AML1, prostate specific

antigen (PSA), PSA-1, PSA-2, PSA-3, prostate-specific membrane antigen (PSMA), T-cell receptor/CD3-zeta chain, and CD20. The cancer antigen may also be selected from the group consisting of MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-Xp2
5 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE-C1, MAGE-C2, MAGE-C3, MAGE-C4, MAGE-C5). In still another embodiment, the cancer antigen is selected from the group consisting of GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, GAGE-9. And in yet a further embodiment, the cancer antigen is selected from the group consisting of BAGE, RAGE, LAGE-1, NAG, GnT-V, MUM-1,
10 CDK4, tyrosinase, p53, MUC family, HER2/neu, p21ras, RCAS1, α-fetoprotein, E-cadherin, α-catenin, β-catenin, γ-catenin, p120ctn, gp100^{Pmel117}, PRAME, NY-ESO-1, cdc27, adenomatous polyposis coli protein (APC), fodrin, Connexin 37, Ig-idiotype, p15, gp75, GM2 ganglioside, GD2 ganglioside, human papilloma virus proteins, Smad family of tumor antigens, lmp-1, P1A, EBV-encoded nuclear antigen (EBNA)-1, brain glycogen
15 phosphorylase, SSX-1, SSX-2 (HOM-MEL-40), SSX-1, SSX-4, SSX-5, SCP-1 and CT-7, and c-erbB-2.

In some important 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.

20 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; aml1; cyclophilin b), B cell lymphoma (Ig-idiotype); Burkitt's (Non-Hodgkin's) lymphoma (CD20); glioma (E-cadherin; α-catenin; β-catenin; γ-catenin; p120ctn), bladder cancer (p21ras), biliary cancer (p21ras), breast cancer
25 (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)--C017-1A/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-1), lung cancer (CEA; MAGE-3; NY-
30 ESO-1), lymphoid cell-derived leukemia (cyclophilin b), melanoma (p15 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-1), ovarian cancer (MUC family; HER2/neu; c-erbB-2), prostate cancer (Prostate Specific Antigen (PSA)

and its immunogenic epitopes PSA-1, PSA-2, and PSA-3; PSMA; HER2/neu; c-erbB-2), pancreatic cancer (p21ras; 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-1),
5 T cell leukemia (HTLV-1 epitopes), and melanoma (Melan-A/MART-1; cdc27; MAGE-3; p21ras; gp100^{Pmel117}). Other cancer antigens are described in U.S. Pat. No. 5,776,427.

The antigens may be administered in a substantially purified form. The term "substantially purified" as used herein refers to a compound which is substantially free of other compounds such as proteins, lipids, carbohydrates or other materials with which it is
10 naturally associated. One skilled in the art can purify viral or bacterial compounds such as polypeptides using standard techniques such as for example protein purification. The substantially pure polypeptide will often yield a single major band on a non-reducing polyacrylamide gel. In the case of partially glycosylated polypeptides or those that have several start codons, there may be several bands on a non-reducing polyacrylamide gel, but
15 these will form a distinctive pattern for that polypeptide. The purity of the viral or bacterial polypeptide can also be determined by amino-terminal amino acid sequence analysis.

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.

20 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
25 activated in vitro and then re-infused into a subject in the activated state. DPP8/9 inhibitors can be combined with the dendritic cells in all of these embodiments. Examples of dendritic cell based vaccines include autologous tumor 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
30 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 second therapeutic agent may also be a hormone therapy agent, particularly for breast and gynecological cancers. DPP8/9 inhibitors can be used in combination with tamoxifen or aromatase inhibitor arimidex (i.e., anastrozole), or simply for disorders responsive to either (e.g., breast cancer).

5 The invention further provides a method for treating a subject having or at risk of developing cancer comprising administering to a subject in need of such treatment an enzyme inhibitor selected from the group consisting of a tyrosine kinase inhibitor, a CDK inhibitor, a MAP kinase inhibitor, and an EGFR inhibitor, and a DPP8/9 inhibitor including a selective or specific DPP8/9 inhibitor in an amount effective to inhibit the cancer. The tyrosine kinase 10 inhibitor may be Genistein (4',5,7-trihydroxyisoflavone), Tyrphostin 25 (3,4,5-trihydroxyphenyl), methylene]-propanedinitrile, Herbimycin A, Daidzein (4',7-dihydroxyisoflavone), AG-126, trans-1-(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, p15, p16, p18, or p19. The MAP kinase inhibitor 15 may be KY12420 ($C_{23}H_{24}O_8$), CNI-1493, PD98059, or 4-(4-Fluorophenyl)-2-(4-methylsulfinyl phenyl)-5-(4-pyridyl) 1H-imidazole. 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 AG1458.

20 DPP8/9 inhibitors 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.

Another form of immunotherapy is the use of lymphokine activated killer cells 25 (LAKs) that are primed in vitro with lymphokines and then re-infused into a subject. The DPP8/9 inhibitors can be combined with such cells either as an addition to the activating lymphokine or in place of it.

The invention also embraces the use of adjuvants. Adjuvant substances derived from microorganisms, such as bacillus Calmette-Guerin, heighten the immune response and 30 enhance resistance to tumors in animals. Adjuvants that may be combined with the DPP8/9 inhibitors include alum, immunostimulatory oligonucleotides such as CpG oligonucleotides, QS-21, and the like.

Other aspects of the invention are directed to treatment of subject having or at risk of developing an infectious disease. A DPP8/9 inhibitor can be administered after viral, bacterial mycobacterial, fungal, or parasitic infection in order to stimulate innate immunity (i.e., immunity mediated by neutrophils, macrophages, NK cells and eosinophils) and/or adaptive immunity (i.e., immunity mediated by T cells and B cells). Alternatively or in addition to, a DPP8/9 inhibitor can be used prophylactically to prevent infection including 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 DPP8/9 inhibition can prime a subject and prepare it for passive exposure to a pathogen. The rate at which a DPP8/9 inhibitor stimulates these cytokines and chemokines (e.g., IL-1β) is useful particularly where pathogen exposure cannot be anticipated.

As used herein, the terms "infectious disease" and "microbial infection" are used interchangeably and intended to convey an infection by any microbe including but not limited to a bacterium, a mycobacterium, a virus, a fungus, a parasite, and the like. The infectious disease may be a bacterial infection, a mycobacterial infection, a viral infection, a fungal infection or a parasitic infection.

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.

Examples of bacterial infections include E. coli, Streptococcal infections, Staphylococcal infections, Pseudomonas infections, Clostridium difficile, Legionella infections, Pneumococcus infection, Haemophilus infections (e.g., Haemophilus influenzae infections), Klebsiella infections, Enterobacter infections, Citrobacter infections, Neisseria infections (e.g., N. meningitidis infection, N. gonorrhoeae infection), Shigella infections, Salmonella infections, Listeria infections (e.g., L. monocytogenes infection), Pasteurella infection (e.g., Pasteurella multocida infection), Streptobacillus infection, Spirillum infection, Treponema inection (e.g., Treponema pallidum infection), Actinomyces infection (e.g., Actinomyces israelli infection), Borrelia infection, Corynebacterium infection, Nocardia infection, Gardnerella infections (e.g., Gardnerella vaginalis infection), Campylobacter infections (e.g., Campylobacter fetus infection), Spirochaeta infections, Proteus infections, Bacteriodes infections, H. pylori, and anthrax.

Examples of viral infections include HIV infection, Herpes simplex virus 1 and 2 infections (including encephalitis, neonatal and genital forms), human papilloma virus infection, cytomegalovirus infection, Epstein Barr virus infection, Hepatitis virus A, B and C infections, rotavirus infection, adenovirus infection, influenza A virus infection, respiratory syncytial virus infection, varicella-zoster virus infections, small pox infection, monkey pox infection, SARS infection, and avian flu virus infection.

In some embodiments, the subject being treated according to the invention is HIV negative.

Examples of fungal infections include candidiasis infection, ringworm, histoplasmosis infection, blastomycosis infections, paracoccidioidomycosis infections, crytococcosis 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
5 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.

DPP8/9 can be used in the treatment of infections regardless of whether the infectious
10 agent produces a deleterious post prolyl cleaving enzyme, as described in US Patent No. 4935493. Thus, in some embodiments, the infectious agent lacks a deleterious post-prolyl cleaving enzyme.

DPP8/9 inhibitors can be used in the treatment of human papillomavirus (HPV) infection. The current therapy for HPV is injection of IFN into a lesion and/or surgical
15 ablation. A systemic treatment of infected subjects with DPP8/9 inhibitors, particularly when administered orally, would be desirable in comparison with current clinical therapies. DPP8/9 inhibitors are similarly useful in combination with HPV vaccines currently in development such as HPV virus-like particle (VLP)-based vaccine (see, for example, Virology 2000 Jan 20;266(2):237-45).

20 A DPP8/9 inhibitor can be administered as a stand alone therapy or in combination with an anti-infective agent (i.e., an anti-microbial). The anti-infective agent may be an anti-bacterial agent, an anti-mycobacterial agent, an anti-viral agent, an anti-fungal agent and an anti-parasitic agent. The anti-infective agent may be an immunotherapeutic agent including antigens, antibodies, cytokines, chemokines, adjuvants and the like.

25 Antigens associated with infectious diseases 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.

A DPP8/9 inhibitor be used with normal and hyper-immune globulin therapy. Normal
30 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
5 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
10 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.

15 The DPP8/9 inhibitor can be used together with an anti-infective that is a chemotherapeutic. 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
20 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;
25 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;
30 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; 5 Cefotiam Hydrochloride; Cefoxitin; Cefoxitin Sodium; Cefpimizole; Cefpimizole Sodium; Cefpiramide; Cefpiramide Sodium; Cefpirome Sulfate; Cefpodoxime Proxetil; Cefprozil; Cefroxadine; Cefsulodin Sodium; Ceftazidime; Ceftibuten; Ceftizoxime Sodium; Ceftriaxone Sodium; Cefuroxime; Cefuroxime Axetil; Cefuroxime Pivoxetil; Cefuroxime Sodium; Cephacetrile Sodium; Cephalexin; Cephalexin Hydrochloride; Cephaloglycin; Cephaloridine; 10 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; 15 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; 20 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 25 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 30 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;
5 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;
10 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;
15 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;
20 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;
25 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;
30 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), Priftin (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 amantidine 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; Foscarnet 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-infectives include Difloxacin Hydrochloride; Lauryl Isoquinolinium Bromide; Moxalactam Disodium; Ornidazole; 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;
5 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
10 Potassium.

The invention also provides a method for preventing drug resistance in a subject. The method involves administering to a subject receiving an anti-microbial agent, a DPP8/9 inhibitor (e.g., a selective or specific DPP8/9 inhibitor) in an amount effective to reduce the risk of resistance to the anti-microbial agent. The subject may be one having or at risk of
15 developing an infectious disease. The subject may have already received an anti-microbial agent, and the infectious disease may have been resistant to such agent. Examples of drug resistant microbes and in some instances the drugs to which they are resistant include Staphylococcus aureus (methicillin), Enterococcus faecium (vancomycin), Streptococcus pneumoniae (penicillin), gonorrhea (penicillin), Acinetobacter baumannii, Escherichia coli,
20 Klebsiella species, Aspergillus, and Pseudomonas aeruginosa.

The invention therefore provides methods for using a DPP8/9 inhibitor (e.g., selective or specific DPP8/9 inhibitor) 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
25 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
30 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.

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 a DPP8/9 inhibitor (e.g., a selective or specific DPP8/9 inhibitor) 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 a DPP8/9 inhibitor (e.g., a selective or specific DPP8/9 inhibitor) 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, a DPP8/9 inhibitor (e.g., a selective or specific DPP8/9 inhibitor) 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-1 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-1 vaccine; 2 total doses recommended); Td: Adult tetanus/diphtheria (2-in-1 vaccine; for use in people over age 7). In another embodiment, a DPP8/9 inhibitor (e.g., a selective or specific DPP8/9 inhibitor) can be used together with oral polio vaccine.

The invention also contemplates treating subjects having or at risk of developing prion diseases. Prion diseases are neurodegenerative diseases that involve aggregates of prion proteins that have an abnormal conformation. Normal prion protein is usually present in the cell membrane of many tissues, particularly neuronal tissue. The abnormally conformed prion protein is believed to be directly involved in converting normally conformed prion protein into more of the abnormally conformed prion protein, which then self-assembles into aggregates that are damaging to neuronal tissue anatomy and function. Some prion diseases are transmissible.

Examples of prion diseases include Creutzfeldt-Jakob disease (CJD), bovine spongiform encephalopathy (BSE), and scrapie. The CJD may be iatrogenic CJD (iCJD), variant CJD (vCJD) or sporadic CJD (sCJD). Subjects at risk of prion diseases include subjects that have ingested "infected" animal products (e.g., a subject that has eaten meat from an animal having "Mad-cow" disease). Further reference to prion diseases, subjects at risk thereof and diagnosis of subjects having prior disease can be found in published PCT Application WO 2004/007743, published January 22, 2004, and these specific descriptions are incorporated by reference herein.

The invention further provides a method for stimulating an immune response in a chronically immunocompromised or immunosuppressed subject comprising administering to a subject in need thereof a DPP8/9 inhibitor (e.g., a selective or specific DPP8/9 inhibitor). Chronic immunosuppression can arise from pharmaceutical use such as the use of deliberate

anti-inflammatories 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, and chronic
5 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. These subjects may be treated according to the invention routinely or only when they are at a higher risk of developing an infectious disease e.g., when traveling to a region where infections are common, when having
10 surgery, when having a skin abrasion, etc.

The invention further provides a method for stimulating an immune response in a genetically immunocompromised subject comprising administering to a subject in need thereof a DPP8/9 inhibitor including a selective or specific DPP8/9 inhibitor. Genetically immunocompromised subjects harbor a genetic mutation that renders them
15 immunocompromised even in the absence of an infectious or exogenous procedure. These subjects include those having a genetic deficiency such as SCID, agammaglobulinemia (e.g., Bruton's agammaglobulinemia and congenital hypogammaglobulinemia), common variable immunodeficiency (CDG), selective immunoglobulin A deficiency, congenital disorder of glycosylation (CDG), or common variable immunodeficiency (CVID).

20 In still other embodiments, the subjects to be treated according to the invention are immunocompetent. Some methods of the invention involve screening of subjects to determine immunocompetency prior to treatment. This can be accomplished by harvesting immune cells (e.g., peripheral blood lymphocytes) from the subject and testing such cells in vitro for responsiveness to stimuli such as mitogens (e.g., PHA or ConA). This can also be
25 accomplished by analyzing the cellular composition of blood, bone marrow, spleen, thymus, lymph node, or other hematopoietic and/or lymphoid tissue in the body of the subject. Those of ordinary skill in the art will be familiar with the normal compositions of these tissues and routinely assess whether the subject appears hematopoietically or immunologically normal.

The invention further provides a method for treating a subject having or at risk of
30 developing an interferon (IFN)-responsive condition. The IFN may be IFNα, IFNα-2b, IFNβ or IFNγ, but is not so limited. The method comprises administering to a subject in need of such treatment a DPP8/9 inhibitor including a selective or specific DPP8/9 inhibitor. The inhibitor may be administered in an amount effective to induce IL-1. The method may further

comprise identification of a subject having or at risk of developing an IFN-responsive condition. In one embodiment, the condition is an IFNγ-responsive condition, including but not limited to viral infections and associated diseases, and cancer. In one embodiment, the IFN-responsive condition is a chronic infection selected from the group consisting of multiple sclerosis, chronic hepatitis B infection, chronic hepatitis C infection, chronic Epstein Barr Virus infection, and tuberculosis. the viral infection may be an HIV infection. Other conditions include hepatocellular carcinoma, Kaposi's Sarcoma (AIDS-related), thick primary melanomas, and regional lymph node metastases. Thus, in one embodiment, the condition is drug resistant.

These methods may further comprise administering to the subject a second therapeutic agent selected from the group consisting of IFNα, pegylated IFN, IFNα-2b, acyclovir, lobucavir, ganciclovir, L-deoxythymidine, clevudine, an antigen or antibody (or fragment thereof) (e.g., hepatitis B-specific immunoglobulin), phosphonoformate (PFA), ribavirin (RBV), thymosin alpha-1, 2 3-dideoxy-3-fluoroguanosine (FLG), famciclovir, lamivudine, adefovir dipivoxil, entecavir, and emtricitabine.

The invention further provides a method for treating a subject having or at risk of developing cardiovascular disease comprising administering to a subject in need of such treatment a DPP8/9 inhibitor (e.g., a selective or specific DPP8/9 inhibitor) in an amount effective to induce an effective amount of IL-1.

In certain embodiments, the invention intends to treat conditions that are not autoimmune diseases such as arthritis or SLE. In other embodiments, the invention does not intend to ameliorate allograft transplantation.

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 and like factors within the knowledge and expertise of the health practitioner. It is preferred generally that a maximum dose be used, that is, the highest safe dose according to sound medical judgment.

For example, in connection with methods for treating subjects having a condition characterized by abnormal mammalian cell proliferation, an effective amount to inhibit the condition 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 population such as, for example, a tumor, and/or to inhibit in whole or in part symptoms associated with the condition. Inhibition can be assessed by the size of a cell mass (e.g., a tumor), or by the presence and/or frequency of cancer cells in the blood or other body fluid or 5 tissue (e.g., a biopsy). As indicated in particular embodiments, some inhibitors are administered in an amount effective to inhibit the condition while reducing in whole or in part side effects such as those associated with post-prolyl cleaving enzymes including Class I inhibitors.

In some embodiments, the DPP8/9 inhibitor is administered in an effective amount to 10 induce apoptosis via activation of caspases 3 and/or 7 in the absence of IL-1beta induction.

Some embodiments of the invention relate to the administration of inhibitors substantially simultaneously. As used herein, the term "substantially simultaneously" means that the inhibitors (or other agents) are administered at the same time or within minutes of each other (e.g., within 10 minutes of each other). The term embraces joint administration as 15 well as consecutive administration. If the administration is consecutive, it is separated in time for only a short period (e.g., the time it would take a medical practitioner to administer two compounds separately). As used herein, concurrent administration and substantially simultaneous administration are used interchangeably.

The invention contemplates a variety of inhibitor administration dosings, schedules 20 and regimens.

The DPP8/9 inhibitor (e.g., the selective or specific DPP8/9 inhibitor) may be administered in a single or in multiple administrations per day. In some important embodiments, the inhibitor is administered in two administrations per day. Any administration per day may include additional inhibitors such as DPP4 and/or FAP inhibitors. 25 One or all administrations per day may include one, two, three or more inhibitor classes. For example, the first administration of a day may include the DPP8/9 inhibitor and the second administration of the day may include the DPP4 and/or FAP inhibitor (optionally with the DPP8/9 inhibitor).

The invention further contemplates administration of a DPP8/9 inhibitor (e.g., a 30 selective or specific DPP8/9 inhibitor) on alternating days. For example, the DPP8/9 inhibitor may be administered on day 2, day 4 and day 6 of a weekly cycle. Alternatively, administration of the DPP8/9 inhibitor may be interrupted for a day or more depending on the

presence and magnitude of side effects. The entire time course may therefore be shifted based on the length of the interruption.

In some instances, a DPP8/9 inhibitor is administered in combination with a DPP4 inhibitor and/or a FAP inhibitor and are administered alone or in combination so as to maximize the therapeutic benefit and minimize the side effect(s) of each, both or all inhibitor classes. In some embodiments, this is accomplished by providing a molar excess of DPP4 inhibitor (including selective or specific DPP4 inhibitor) and/or a FAP inhibitor (including selective or specific FAP inhibitor) relative to a DPP8/9 inhibitor (including a selective or specific DPP8/9 inhibitor). For example, the molar ratio of DPP8/9 inhibitor to DPP4 and/or FAP inhibitor can be 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, 1:90 or 1:100. The molar ratio as used herein refers to the total moles of the DPP8/9 inhibitor:total moles of DPP4 and/or FAP inhibitor. The DPP4 and/or FAP inhibitor can also be administered in a greater than 100 molar excess (as compared to the DPP8/9 inhibitor) (e.g., the DPP8/9 inhibitor: DPP4 and/or FAP inhibitor molar ratio is 1:150, 1:200, etc.).

The invention further embraces the administration of different inhibitors at different times. For example, a DPP8/9 inhibitor (e.g., a selective or specific DPP8/9 inhibitor) may be administered before, before and during, before and after, during and after, or before, during and after administration of a DPP4 inhibitor and/or a FAP inhibitor. In still other embodiments, a DPP4 inhibitor and/or a FAP inhibitor is administered before a DPP8/9 inhibitor (e.g., a selective or specific DPP8/9 inhibitor).

In yet other embodiments, the inhibitors are administered in an alternating manner. For example, the DPP8/9 inhibitor (e.g., the selective or specific DPP8/9 inhibitor) is administered first, followed by the DPP4 inhibitor and/or the FAP inhibitor, followed by the DPP8/9 inhibitor, followed by the DPP4 inhibitor and/or the FAP inhibitor, etc. More specifically, the DPP8/9 inhibitor may be administered on days 1, 3, 5, 7, etc. and the DPP4 inhibitor and/or the FAP inhibitor may be administered on days 2, 4, 6, 8, etc. In another embodiment, the DPP8/9 inhibitor is administered on a number of days (e.g., 2, 3, 4, 5, 6, 7, or more days), followed by administration of the DPP4 inhibitor and/or the FAP inhibitor for a number of days (e.g., 2, 3, 4, 5, 6, 7, or more days). The number of days in which the DPP8/9 inhibitor is administered may be equal to or different from the number of days in which the DPP4 inhibitor and/or the FAP inhibitor is administered.

The timing of administration may also depend upon whether a second therapy is used in combination with the DPP8/9 inhibitor (e.g., the selective or specific DPP8/9 inhibitor). Thus, for example, in some embodiments relating to for example cancer therapy the inhibitor is used in combination with a chemotherapeutic or an immunotherapeutic agent that is
5 administered on day 1 of a treatment cycle. The inhibitor may be administered on days 2-7 of the same treatment cycle. The cycle may be 7, 14, 21, 28, 35, 42, 49 days or longer. The cycle may be performed once, twice, three times, four times, or more, optionally with rest periods (e.g., days or weeks in which no therapy is administered) in between. As an example, the cycle may be a 21 day cycle in which the immunotherapeutic or chemotherapeutic agent is
10 administered on days 1 and 8, the inhibitor is administered on days 2-7 and 9-14, and days 15-21 are rest days. As another example, the cycle may be a 21 day cycle in which the immunotherapeutic or chemotherapeutic agent is administered on days 1, 8 and 15, and the inhibitor is administered on days 2-7, 9-14 and 15-21.

The DPP8/9 inhibitor (e.g., the selective or specific DPP8/9 inhibitor) may be
15 administered prior to the administration of the chemotherapeutic or immunotherapeutic agent, with administration of the DPP4 inhibitor (e.g., the selective or specific DPP4 inhibitor) and//or the FAP inhibitor (e.g., the selective or specific FAP inhibitor) during and/or after administration of the chemotherapeutic or immunotherapeutic agent.

A "routine schedule" as used herein, refers to a predetermined designated period of
20 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
25 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
30 administration on a certain day.

The agents may be formulated separately or together with each other or with secondary therapeutic agents. Being formulated together means that the agents are present in the same composition prior to administration to the subject. Being formulated separately

means the agents are present in separate and distinct compositions prior to administration to the subject. In this latter instance, however, they may be commingled immediately prior to administration to the subject.

The inhibitor can be administered to a subject by any route that delivers the inhibitor to the affected site, either directly or indirectly. Preferred routes of administration include but are not limited to oral, topical (including intranasal, ocular, vaginal, rectal, transdermal, etc.), and parenteral (including intramuscular, intravenous, subcutaneous, etc.), by inhalation and intratracheal. Delivery may be local (e.g., mucosal) or systemic. The administration route of the inhibitor(s) and other therapeutic agents may be the same or it may be different. In some embodiments, the inhibitors are administered orally, and the other therapeutic agent is administered by a non-oral route.

The invention provides pharmaceutical compositions. Pharmaceutical compositions are sterile compositions that comprise effective amounts of active agent, such as the inhibitors of the invention, preferably in a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier" means one or more compatible solid or liquid filler, diluents or encapsulating substances which are suitable for administration to a human or other subject contemplated by 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 are commingled in a manner that precludes interaction that would substantially impair their desired pharmaceutical efficiency.

The inhibitors (and secondary therapeutic agents) may be administered per se (neat) or in the form of a salt. The salts are preferably pharmaceutically acceptable. Salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulphonic, tartaric, citric, methane sulphonic, formic, malonic, succinic, naphthalene-2-sulphonic, and benzene sulphonic. Salts can also be alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group.

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).

The compounds, 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. Pharmaceutical parenteral formulations include aqueous solutions of the active ingredients in water-soluble form. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Alternatively, suspensions of active ingredients may be prepared as oil-based suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. A suitable compound for sustained release delivery is GELFOAM, a commercially available product consisting of modified collagen fibers.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

The active agents may be formulated in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

For oral administration, the agents can be formulated readily by combining 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. Pharmaceutical compositions for oral use can be obtained as solid excipients, 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 compositions 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 active compounds 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 compositions may take the form of tablets, lozenges, gum, dissolvable sheets or films, pastes (such as tooth pastes), washes (such as mouth washes), formulated in conventional manner.

For administration by inhalation, the compounds 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 compounds 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.

The pharmaceutical compositions may also be formulated for topical delivery (e.g., to 5 the skin) by commingling the active ingredients with bases such as creams, ointments or lotions.

In addition to the formulations described previously, the compounds 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) 10 or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

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 15 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 20 instant invention, the agent 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 25 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 may be further 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 30 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 can be used to deliver agents to the subject. Biodegradable matrices are preferred. Such polymers may be natural or
5 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
10 multivalent ions or other polymers.

In general, agents 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
15 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,
20 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),
25 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.

30 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
5 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, C.P. Pathak and J.A. Hubell in Macromolecules, 1993, 26, 581-587, the teachings of which are incorporated herein, polyhyaluronic acids, casein, gelatin, glutin, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl
10 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 inhibitors for use as a medicament, methods for preparing the medicament, and methods for the sustained
15 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 agent, 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
20 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
25 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 5,736,152 and (b)
30 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.

0

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 delivery therapeutic levels of the active ingredient for at least 30 days, at least 60 days and more
5 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.

For a brief review of methods for drug delivery, see Langer, *Science* 249:1527-1533, 1990, which is incorporated herein by reference.

The invention further provides kits that minimally comprise a DPP8/9 inhibitor
10 optionally formulated for oral administration in one container. In one embodiment, the kit contains another container having antibody or antibody fragment, preferably formulated for administration by injection. In another embodiment, the kit may comprise another container having an antigen, or a cocktail of antigens. 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
15 prior to administration to a subject. All the kits of the invention can optionally contain instructions for storage, reconstitution (if applicable) and administration.

The following Examples are provided to illustrate specific instances of the practice of the present invention and are not intended to limit the scope of the invention. As will be
20 apparent to one of ordinary skill in the art, the present invention will find application in a variety of compositions and methods.

Examples

**Example 1. Assays for inhibition of the dipeptidyl peptidase activity of DPP4, DPP8/9,
25 FAP and DPP2 by amino boronic dipeptide, cyanopyrrolidine and isoindoline compounds**

Materials and Methods.

Production of soluble DPP4 enzyme. Production of plasmid DNA encoding soluble
30 DPP4 was carried out essentially as described in published PCT application WO 2005/071073 published August 4, 2005. Plasmids encoding soluble recombinant DPP4 were introduced into 293T epithelial cells (CRL-11268, ATCC) by transient transfection. These cells express little or no DPP4 endogenously. 293T cells were plated at 6 x 10^6 in 10 cm plates one day

before transfection. Cells were transfected with 10 or 20 μg purified plasmid DNA using Lipofectamine 2000 (InVitrogen) according to manufacturer's instructions. After overnight incubation with Lipofectamine/ DNA, the tissue culture medium was changed to Freestyle 293 Expression medium (serum free, InVitrogen) containing penicillin and streptomycin.
5 Supernatant was harvested at 4-6h intervals (6 ml) or after overnight incubation (10 ml). Supernatant containing secreted soluble recombinant DPP-IV was centrifuged, sterile filtered, and stored at 4°C. Supernatants were used directly as a source of DPP-IV.

Recombinant human DPP 8. DPP8 cDNA was obtained by PCR of cDNA derived from a Caco2 cell line using KOD XL polymerase using 2 primer pairs: DPP8-Sfi (5' GTA
10 GTC GGCC CAGCC GGCC ATG GCA GCA GCA ATG GAA ACA GAA-3' SEQ ID NO: 4) and DPP8-R (5' GTAGTCA GCGGCCGC TCA TGA GGC TTG TAG AGC ATC CCA TAC AAT GT-3' SEQ ID NO: 5), and DPP8-Sfi and DPP8-R2 (5' GTAGTCA GCGGCCGC TTA TAT CAC TTT TAG AGC AGC AAT ACG TGA TCC AAG 3' SEQ ID NO: 6). The PCR product was digested with SfiI and NotI and the SfiI-NotI fragment cloned
15 in pSecTag-2B. This placed the DPP8 downstream of the resident secretion sequence in pSecTag-2B. The complete coding sequence was obtained in stages by splicing together sections with no PCR errors to give an intermediate plasmid (#187). Finally, the 5' section was then removed with NheI (which cut upstream of the secretion sequence in pSecTag-2B, and inside DPP8) and replaced by a 780 nt 5' NheI PCR fragment which deleted the secretion
20 sequence to give plasmid #197 so that wild-type DPP8 could be expressed. The primer pair DPP8-cyto 5'-CCAAGCTG GCTAGC TCA AAC AGA CACCATG GCAGCAGCAATGGAAACAGAA-3' SEQ ID NO: 7) and DPP8-R were used in this procedure.

A second plasmid (#237) expressing a DPP8-myc- 6xHis fusion protein was made by
25 replacing a section at the 3' end of plasmid #197 with a PCR product in which the stop codon was deleted and an ApaI site placed to give in frame fusion with the tag sequence in pSecTag-2B. In summary, two plasmids were made in pSecTag-2B vector (Invitrogen); the first contained wild-type DPP8 sequence (plasmid #197), the second was a myc 6xHis fusion at the C-terminus of DPP8 (plasmid #237).

30 **Recombinant human DPP9.** DPP9 cDNA was obtained by PCR using oligo dT primed cDNA from RNA isolated from human bone marrow stromal cells. KOD XL polymerase was used with the PCR primer pair: DPP9-cyto (5'-CCA AGC TGTCTAGATC AAACAGACACCATG GCCACCACCGGGACCCCAACGGCCGA-3' SEQ ID NO: 8) and

DPP9-R (5'-GTAGTCA GCGGCCGC TCA GAG GTA TTC CTG TAG AAA GTG CAG CAA CGT-3' SEQ ID NO: 9). The 5' primer introduced a XbaI site which is compatible with the NheI site in the pSecTag-2B vector. Note that an internal NheI site in DPP9 made NheI unusable. The resulting PCR product was isolated and digested with XbaI and NotI restriction
5 enzymes. The 2.6 kb fragment was isolated by gel electrophoresis under standard conditions (0.8% agarose, TAE) and ligated to NheI and NotI digested pSecTag-2B vector.

DNA sequence of a plasmid (#392) prepared from one of the ampicillin-resistant transformants contained the appropriate size insert, and was determined to encode the correct DPP9 primary sequence. Three silent PCR-induced mutations were observed.

10 **Recombinant PEP.** PEP encoding cDNA was obtained by PCR of oligo dT primed cDNA from RNA isolated from human bone marrow stromal cells. KOD XL polymerase was used with the primer pair: PEP-F (5'-CCA AGC TGG GCT AGC CAA ACA GAC ACC ATG CTG TCC CTT CAG TAC CCC GAC GTG-3' SEQ ID NO: 10) and PEP-tag (5'-GTA GTC AGC GGG CCC TGG AAT CCA GTC GAC GTT CAG GCA CCG-3' SEQ ID NO:
15 11). The PCR program was 94°C, 40 sec followed by 30 cycles of 94°C for 15 sec, 54°C for 20 sec, and 62 °C for 4 min. A NheI restriction site was placed upstream of the ATG start codon underlined in primer sequence above, and an ApaI site was placed at the end of the coding sequence to give an in phase fusion with the myc and 6xHis tags in the pSecTag-2B vector (InVitrogen). The PCR product was isolated and digested with NheI and ApaI
20 restriction enzymes. The 2.2 kb fragment was isolated by standard gel electrophoresis (0.8% agarose,TAE) and ligated to NheI and ApaI digested pSecTag-2B vector.

DNA sequence of a plasmid (#409) prepared from one of the ampicillin-resistant transformants contained the appropriate size insert and was determined to encode the correct PEP primary sequence with no PCR-induced mutations.

25 **Production of soluble DPP8, DPP9 and PEP enzymes.** 293T cells were transfected with the appropriate recombinant plasmid using Lipofectamine 2000 (InVitrogen). 5-6 x 10^6 cells were cultured in a 10 cm dish one day prior to transfection. Culture medium was DMEM, 10% fetal calf serum, penicillin, streptomycin and glutamine (all at standard concentrations). Cells were transfected with 12 ug DNA and 30 µl Lipofectamine reagent in
30 1.5 ml Optimem I and 9 ml fresh antibiotic-free medium was added to each 10 cm dish. After 24h, medium was aspirated and the dishes of adherent cells frozen at -80°C. It was determine that active enzymes could be obtained from the cells after at least 1 year of frozen storage. Enzyme was produced as a Triton-X100 extract of the cells by adding 3.5 ml cold lysis buffer

(25 mM Tris-Cl pH 7.5, 1% Triton-X100, 150 mM NaCl) directly to the adherent cells. Cells were collected by scraping with a cell scraper and transferred to a 15 ml tube and incubated 30 min. on ice to obtain complete lysis. Lysate was cleared by centrifugation for 5 min. at >10,000xg, and aliquots frozen at -80°C. Frozen extracts maintained specific enzyme activity for at least 1 year. In addition, recombinant human DPP-8 containing six histidine residues at the C-terminus was expressed as an intracellular fusion protein in 293T cells and purified from cellular extracts by nickel affinity chromatography. Eight tissue culture plates (150 mm wells) of transfected cells were lysed in 20 ml M-Per (Pierce) following the manufacturer's instructions. The extract was affinity purified on 2 nickel columns using the *B-Per 6XHis Fusion Protein Purification Kit* (Pierce). Material was eluted from the columns in a 4 mL fraction of immidazole buffer according to the manufacturer's instructions. Purified enzyme was diluted 120-fold in HEPES saline assay buffer (pH 8.1) containing 0.1% BSA.

Recombinant, soluble human FAP. A truncated soluble recombinant FAP was made from a cDNA clone of human FAP and fused to a secretion signal in the secretion vector pSecTag 2B. This construct allowed production of soluble FAP in 293T mammalian cells. The strategy was based on the N-terminal sequence of serum DPP IV (see above) and was designed to produce a truncated FAP in which a signal/leader sequence is joined to the residue in FAP analogous to the amino-terminus of serum DPP IV. The transmembrane sequence was deleted in order to render the recombinant FAP soluble. The sequence of the amino terminus of human serum DPP IV and the analogous site for FAP are as follows. The transmembrane anchors of the naturally occurring integral membrane proteins are underlined.

```
hDPP IV:              MKTPWKVLLGLLGAAALVTIITVPVVLLNKGTDDATADSRKTYTLTDYLKN-
(SEQ ID NO: 12)
Serum DPP4*:                                                    SRKTYTLTDYLKN-
(SEQ ID NO: 13)
                                                                 RKTYTLTDYLKN-
(SEQ ID NO: 14)

hFAP:                  MKTWVKIVFGVATSAVLALLVMCIVLRPSRVHNSEENTMRALTLKDILNG-
(SEQ ID NO: 15)
Chosen N-terminus:                                              TMRALTLKDILNG-
(SEQ ID NO: 16)
```

In the recombinant secreted FAP the leader sequence was provided by the secretion plasmid vector pSecTag2-B (InVitrogen) in which an immunoglobulin kappa light chain secretion signal is situated upstream of a polylinker allowing the insertion of human or mouse

FAP cDNA in phase. The SfiI restriction site in the vector was chosen in order to minimize the number of residues contributed by the vector in the N-terminal portion of recombinant FAP as shown below.

```
pSecTag2 vector (InVitrogen):
                                                                  | SfiI site
932 GTA CTG CTG CTC TGG GTT CCA GGT TCC ACT GGT GAC GCG GCC CAG CCG
    Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly|Asp Ala Ala Gln Pro
                                               |Signal cleavage site
(SEQ ID NOS: 17 AND 18, respectively)
```

Mature FAP contained 6 residues derived from the vector. The DNA sequence of the vector-FAP junction encoding the 6 vector-derived residues DAAQPA included in mature secreted FAP was as follows.

```
5' ggt/gac gcg gcc cag ccg gcc ACAAAGAGAGCTCTTACCcTGAAGGATATTTTAAATG 3'
   g  /D   A   A   Q   P   A  -T  M  R  A  L  T  L  K  D  I  L  N  G
      /---------Vector--------|-----FAP--- >
(SEQ ID NOS: 19 AND 20, respectively)
```

This recombinant FAP also contains a single mutation at threonine 229 (T229M mutation) and therefore lacks one of the six glycosylation sites due to destruction of the N-x-T glycosylation motif at N227.

Production of soluble human FAP. Soluble recombinant FAP was produced by transient transfection of 293T cell (CRL-11268, ATCC) epithelial cells that do not express FAP endogenously. 293T cells were plated at 6 x 10^6 in 10 cm diameter plates one day before transfection. Cells were transfected with 10 or 20 μg purified plasmid DNA using Lipofectamine 2000 (InVitrogen) according to manufacturer's instructions. After overnight incubation with Lipofectamine/ DNA, the tissue culture medium was changed to Freestyle 293 Expression medium (serum free; InVitrogen) containing penicillin and streptomycin. Culture supernatant was collected 66-72 hours after transfection and frozen prior to purification on MacroPrep High Q Support (BioRad) ion exchange resin. A total of 1.5 litres of expression medium containing secreted rhFAP was diluted in 50 mM Tris, pH 8.1 to give a NaCl concentration of 75-100 mM. The diluted material was run over a 5 mL High Q column, the column washed with 50 mM Tris, 75 mM NaCl, pH 8.1, and eluted with 50 mM Tris, 400 mM NaCl, pH 8.1. Material that initially flowed through the column was reapplied, washed and eluted. Reapplication of eluted material was performed twice. The pooled eluted material

was diluted 3-fold in 50 mM Tris, pH 8.1 to give a final salt concentration of approximately 133 mM, and concentrated approximately 75-fold using a Centricon Plus-80 concentrator (Millipore). Purified enzyme was diluted 267-fold in HEPES saline assay buffer (pH 8.1) containing 0.1% BSA.

5 **Human DPP2.** Soluble DPP2 purified from human placenta was purchased from Enzyme Systems Products (Livermore, CA).

Determination of IC_{50} values for inhibition of dipeptidyl peptidase activity of amino boronic dipeptide and cyanopyrrolidine compounds. Each dipeptidyl peptidase was preincubated for 5 min. at room temperature with the amino boronic dipeptides L-valinyl-
10 L-boroproline (talabostat [PT-100]), L-glutamyl-L-boroproline, L-isoleucyl-L-boroproline, or L-norleucyl-L-boroproline, or the cyanopyrrolidine compounds 1-[2-[5-cyanopyridin-2-yl)amino]ethylamino]acetyl-2-cyano-(*S*)-pyrrolidine (cyanopyrrolidine 1) or 1-[[(3-hydroxy-1-adamantyl)amino]acetyl]-2-cyano-(*S*)-pyrrolidine (cyanopyrrolidine 2) or with no addition. Each compound was added at a concentration of 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} or 10^{-10} M.
15 After preincubation, the fluorogenic substrate Ala-Pro-7-amino-4-trifluoro-methyl coumarin (Ala-Pro-AFC, Enzyme Systems Products was added at a final concentration of 0.4 mM, and the reaction mixture incubated for a further 15 min. at room temperature. Reactions were stopped by addition of 1 M sodium acetate and fluorescence was measured in a SpectroMax Gemini XS fluorometer (Molecular Devices, Sunnyvale, CA) using an excitation wavelength
20 of 400 nm and an emission wavelength of 505 nm. It should be noted that the reaction mixtures were buffered to the optimal pH for each dipeptidyl peptidase as follows: FAP and DPP 8, pH 8.1; DPP 4, pH 7.6; DPP 2, pH 5.5. IC_{50} values were determined from enzyme-inhibition curves obtained by plotting fluorescence units against talabostat concentration.

Determination of IC_{50} values for inhibition of dipeptidyl peptidase activity or
25 **isoindoline compounds PTX-1200 and PTX-1210.** Isoindoline inhibitors[2] with a (S)-cyclohexylglycine (PTX-1200) or a 1-(4,4'-difluor-benzhydryl)-piperazine (PTX-1210) at the P2 site were dissolved, respectively, as a 0.07M stock in 50% DMSO or as 0.1M in 100% DMSO. Secondary 1 mM stocks were made in 1mM HCl for routine use. Inhibitors were stored at -20°C. Dilutions for inhibition assays were in water.

30 90 µl of each enzyme preparation diluted in HEPES/NaCl (pH 8.1) assay buffer (50 mM HEPES, 140 mM NaCl, 0.1% bovine serum albumin) was added to the wells of a 96-well black-sided assay plate and incubated at 32°C for >10 min. 5 µl of inhibitor compound at a concentration 20-fold higher than the final concentration in complete reaction mixture was

added, and the plate incubated for 5 min to allow inhibitor binding. The enzyme reaction was then started by addition of 5 µl of 8 mM Ala-Pro-AFC to provide a 0.4 mM final concentration. Plates were placed in a SpectroMax Gemini XS fluorometer (Molecular Devices, Sunnyvale, CA) and incubated at 32°C. Fluorescence was measured (excitation
5 wavelength of 400 nm and emission wavelength of 505 nm) every 42 sec for 15 min. Enzyme reaction rates were derived by linear regression from the linear portion of the curves and expressed as fluorescence units per sec. IC_{50} values were obtained by manual interpolation from the curves.

10

Results.

IC_{50} values for amino boronic dipeptide and cyanopyrrolidine compounds.

Table 1. IC_{50} values of amino boronic dipeptides and cyanopyrrolidine compounds for inhibition of dipeptidyl peptidase cleavage of Ala-Pro-AFC

Compound	Point No.	IC_{50} (nM) for inhibition of dipeptidyl peptidase:					
		DPP 8	DPP 9	DPP-IV	DPP 2	FAP	PEP
Val-boroPro	PT-100	3	3	1	30	32	50
Ile-boroPro	PT-510	4	4	2	22	30	47
NorLeu-boroPro	PT-790	2	NT	1	1	23	NT
Glu-boroPro	PT-630	27	25	2	22	23	850
Cyanopyrrolidine 1	None assigned	4,000	NT*	7	20,000	12,000	NT
Cyanopyrrolidine 2	None assigned	4,500	NT	15	>100,000	30,000	NT

*Not tested

15

IC_{50} values for isoindoline compounds.

Table 2. IC_{50} values of isoindoline compounds for inhibition of dipeptidyl peptidase cleavage of Ala-Pro-AFC

Compound	Point No.	IC_{50} (nM) for inhibition of dipeptidyl peptidase:					
		DPP 8	DPP 9	DPP-IV	DPP 2	FAP	PEP
	PTX-1200	18	26-45	30000	30000	>50000	>50000
	PTX-1210	25	105	>50000	>50000	>50000	>50000

*Not tested

Comparison of IC_{50} values indicates that the amino boronic dipeptides are relatively
20 non-selective inhibitors of the dipeptidyl peptidase activity of the serine proteases classified as members of the DPP-IV family.[3] The related post-prolyl cleaving enzyme PEP was also sensitive to inhibition by the amino boronic dipeptides that were tested for inhibition of this enzyme (PT-100 and Ile-boroPro). The data are shown in Table 1. Val-boroPro, Ile-boroPro and NorLeu-boroPro inhibited DPP 8, DPP 9, and DPP-IV with IC_{50}s in the range of 1 to 4
25 nM. IC_{50}s were approximately 10-fold higher for DPP 2, FAP and PEP with the exception of an IC_{50} of 1 nM for inhibition of DPP 2 by NorLeu-boroPro. Glu-boroPro was also a non-

selective inhibitor of DPP 8, DPP 9, DPP-IV, DPP 2 and FAP; but, compared to the other amino boronic dipeptides, it was a relatively weak inhibitor of PEP. In marked contrast, the cyanopyrrolidine compounds 1 and 2 were shown to be selective inhibitors of DPP-IV, and the isoindolines PTX-1200 and PTX1210 to be extremely selective inhibitors of DPP 8 and 5 DPP 9 (Table 2).

References.

1. Durinx C, Lambeir AM, Bosmans E, Falmagne JB, Berghmans R, Haemers A, Scharpe S, De Meester I. Molecular characterization of dipeptidyl peptidase activity in serum: 10 soluble CD26/dipetidyl peptidase IV is responsible for the release of X-Pro dipeptides. Eur. J. Biochem. 2000;267

2. Jiaang WT, Chen YS, Hsu T, Wu SH, Chien CH, Chang CN, Chang SP, Lee SJ, Chen X. Novel isoindoline compounds for potent and selective inhibition of prolyldipeptidase DPP8. Bioorg. Med. Chem. Lett. 2005;15:687-691

15 3. Gorrell MD. Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. Clin. Sci. 2005;108:277-292

<u>**Example 2.**</u>

Materials and Methods.

20 **Cell lines, cell culture and conditioned medium.** The THP-1 human monocytic cell line (American Type Culture Collection, Manassas, VA) was propagated in RPMI 1640 (Life Technologies, Inc., Rockville, MD) supplemented with heat-inactivated fetal bovine serum (10%), HEPES (10 mM), glutamine (1 mM), penicillin (100 units/ml), streptomycin (100 μg/ml), and this medium was also used for the caspase-1 and IL-1β induction assay cultures. 25 Where indicated, THP-1 cells were differentiated prior to caspase-1 activation and IL-1β induction assays by incubation (37°C) in 5 μg/ml phorbol myristate acetate (PMA, Sigma, St. Louis, MO) for 3 hours. Conditioned medium was obtained from cultures of MM45T.Sp cells (American Type Culture Collection) in fully supplemented RPMI 1640 and fractionated by Amicon Centriplus YM-3 (Millipore, Billerica, MA) filtration to produce a filtrate containing 30 material of ≤3kDa in molecular size. The filtrate was reconstituted with heat-inactivated fetal bovine serum (10%) and is referred to as ≤3kDa factor.

Caspase-1 activation assays. 5 x 10^4 PMA-differentiated THP-1 cells were added to the wells of black 96-well plates (Corning Inc., Life Sciences, Acton, MA) and incubated for

18 hours. The compounds PT-100, PTX-1200 or PTX-1210 and the ≤3kDa factor were then added to the culture wells. After incubation (37°C) for 2 hours, the supernatant medium of the cultures was replaced with 0.2 ml PBS-D containing 50 μM N-acetylated Trp-Glu-His-Asp-7-amino-4-trifluoro-methyl coumarin (Ac-WEHD-AFC, Enzyme Systems, Livermore, CA).
5 From 120 to 250 minutes after the addition of compounds fluorescence was monitored in a SpectroMax Gemini XS fluorometer (Molecular Devices, Sunnyvale, CA) using an excitation wavelength of 400 nm and an emission wavelength of 505 nm. For each compound the assays was performed in triplicate culture wells and mean fluorescence (± standard deviation [SD]) was calculated

10 Caspase-1 activation was also investigated in THP-1 cells that were not differentiated by PMA treatments. Undifferentiated THP-1 cells were added to wells of black 96-well plates in PBS-D together with PT-100 or PTX-1210 and Ac-WEHD-AFC at the beginning of the experiment. From 10 to 243 minutes after the beginning of the experiment fluorescence was monitored and the data calculated as described above.

15 **IL-1β induction assays.** 2.5 x 10^5 PMA-differentiated THP-1 cells were added to wells of 24-well plates (Corning Inc., Life Sciences, Acton, MA) and after incubation for 18 hours the compounds PT-100, PTX-1200 or PTX-1210 with ≤3kDa factor were added to the culture wells. Supernatant concentrations of IL-1β were determined in duplicate by Quantikine ELISA (R&D Systems, Minneapolis, MN) at 120, 180 and 240 minutes after
20 addition of compounds.

Results.

The post-proline dipeptidyl aminopeptidases DPP8[2] and DPP9[3,4] are members of the DPP-IV gene family that are expressed as soluble proteins localized in the cytoplasm.[5] Both
25 DPP8 and DPP9 are expressed ubiquitously in human tissues and in leucocytes.[5] Talabostat inhibits all the enzymatically active members of the DPP4 gene family: DPP4 itself, DPP2, DPP8, DPP9 and FAP. Therefore, in order to investigate the role of DPP8 and DPP9 in the induction of IL-1β by talabostat in monocytes, the activities of the DPP8/9-selective isoindoline compounds PTX-1200 and PTX-1210 were compared with the activity of
30 talabostat in cultures of the THP-1 human monocytic cell line. DPP 8 and DPP 9 are closely related, and PTX-1200 and PTX-1210 inhibit these dipeptidyl peptidases with IC_{50} values in range of 25 to 105 nM, whereas IC_{50} values for inhibition of DPP-IV, FAP, and DPP 2 are greater than 30,000 nM. THP-1 cells are similar to primary monocytes, and they

constitutively express high levels of inactive pro-caspase-1 but not IL-1β in either its immature (pro-IL-1β) or mature form.[6] THP-1 cells can be stimulated to differentiate into adherent cells that express cytoplasmic pro-IL-1β by incubation with phorbol myristate acetate (PMA).[7] Talabostat at a concentration of 10 μM was shown to stimulate IL-1β secretion by THP-1 cells that had been differentiated by prior PMA treatment (FIG. 1B). Secreted IL-1β could be detected in the supernatant of cultured cells at 120 minutes after addition of talabostat, and the supernatant IL-1β concentration increased steadily over a 4-hour incubation period. Likewise, both PTX-1200 and PTX-1210 at the 10 μM concentration stimulated IL-1β secretion with similar kinetics (FIG. 1B).

The secretion of IL-1β by monocytes is critically dependent upon the activity of the cytoplasmic cysteine protease caspase-1, also known as IL-1β converting enzyme or ICE.[8] Caspase-1 cleaves the 31 kDa pro-form of IL-1β to produce biologically active, 17 kDa mature IL-1β which is secreted from the monocyte by a pathway that does not involve the classical endoplasmic reticulum to golgi pathway.[9] Caspase-1 itself is expressed as an inactive 45-kDa zymogen that requires cleavage to form an enzymatically active complex of 10-kDa and 20-kDa chains that can process pro-IL-1β. Activation of caspase-1 appears to occur within a multicomponent protein complex called the inflammasome; but little is known about the biochemical interactions that regulate inflammasome formation and caspase-1 activation within the monocyte.[10] The possible role of DPP 8 or DPP 9 in caspase-1 regulation was investigated in THP-1 cells using the selective inhibitors. Talabostat, PTX-1200 and PT-1210 were all shown to activate caspase-1 by addition of the synthetic, fluorogenic caspase-1 substrate Ac-WEHD-AFC to the cultures 2 hours after addition of the compounds (FIG. 1A). The kinetics of caspase 1 activation were similar for all 3 compounds and also resembled the kinetics of IL-1β secretion (FIG. 1). In this experiment, the responses of PMA-differentiated THP-1 cells were obtained in the presence of the ≤3kDa filtrate of conditioned medium from cultures of the MM45T.Sp fibroblastic cell line because this material contains a factor that appears to be required for IL-1β secretion stimulated by talabostat or the DPP 8/9 selective compounds. However, in a second experiment it was shown the neither PMA-differentiation nor the ≤3kDa factor was required for caspase-1 activation by talabostat, or PTX-1210 (FIG. 2), thereby demonstrating the DPP 8/9 inhibition is sufficient to activate caspase-1 in monocytic cells. The data indicate that caspase-1 activation can result from the inhibition of DPP 8 and/or DPP 9. Importantly, inhibition of DPP 8 and/or DPP 9 appears to be the critical event in the IL-1β dependent immunostimulatory mechanism of action of talabostat.

References.

1. Jiaang WT, Chen YS, Hsu T, Wu SH, Chien CH, Chang CN, Chang SP, Lee SJ, Chen X. Novel isoindoline compounds for potent and selective inhibition of prolyldipeptidase DPP8. Bioorg. Med. Chem. Lett. 2005;15:687-691

2. Abbott CA, Yu DMT, Woollatt E, Sutherland GR, McCaughan GW, Gorrell MD. Cloning, expression and chromosomal localization of a novel human dipeptidyl peptidase (DPP) IV homolog, DPP8. Eur. J. Biochem. 2000;267:6140-6150

3. Ajami K, Abbott CA, McCaughan GW, Gorrell MD. Dipeptidyl peptidase 9 has two forms, a broad tissue distribution, cytoplasmic location and DPIV-like peptidase activity. Biochim. Biophys. Acta. 2004;1679:18-28

4. Olsen C, Wagtmann N. Identification and characterization of human Dpp9, a novel homologue of dipeptidyl peptidase IV. Gene. 2002;299:185-193

5. Gorrell MD. Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. Clin. Sci. 2005;108:277-292

6. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-1β. Mol. Cell. 2002;10:417-426

7. Tsuchiya S, Kobayashi Y, Goto Y, Okumura H, Nakae S, Konno T, Tada K. Induction of maturation in cultured human monocytic leukemia cells by phorbol diester. Cancer Res. 1982;42:1530-1536

8. Thornberry NA, Bull HG, Calaycay JR, Chapman KT, Howard AD, Kostura MJ, Miller DK, Molineaux SM, Weidner JR, Aunins J, al e. A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. Nature. 1992;356:768-774

9. Rubartelli A, Sitia R. Secretion of mammalian proteins that lack a signal sequence. In: Kuchler K, Rubartelli A, Holland BI, eds. Unusual Secretory Pathways: from bacteria to man. Austin: R.G. Landes; 1997:87-104

10. Martinon F, Tschopp J. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory disease. Cell. 2004;117:561-574

Equivalents

It should be understood that the preceding is merely a detailed description of certain embodiments. It therefore should be apparent to those of ordinary skill in the art that various modifications and equivalents can be made without departing from the spirit and scope of the invention, and with no more than routine experimentation.

5 All references, patents and patent applications that are recited in this application are incorporated by reference herein in their entirety, unless otherwise stated.

What is claimed is:

Claims

1. A method for stimulating an immune response comprising
administering to a subject in need thereof a selective DPP8/9 inhibitor in an amount effective to stimulate an immune response,
wherein the selective DPP8/9 inhibitor inhibits DPP8/9 to a greater extent than it inhibits at least DPP4.

2. A method for treating a subject having or at risk of developing a condition characterized by abnormal cell proliferation comprising
administering to a subject in need thereof a selective DPP8/9 inhibitor in an amount effective to inhibit the condition,
wherein the selective DPP8/9 inhibitor inhibits DPP8/9 to a greater extent than it inhibits at least DPP4.

3. A method for treating a subject having or at risk of developing a condition characterized by abnormal cell proliferation comprising
administering to a subject in need thereof a selective DPP8/9 inhibitor and a DPP4 inhibitor in an amount effective to inhibit the condition,
wherein the selective DPP8/9 inhibitor inhibits DPP8/9 to a greater extent than it inhibits at least DPP4.

4. The method of claim 3, further comprising administering a fibroblast activation protein (FAP) inhibitor to the subject.

5. A method for treating a subject having or at risk of developing a condition characterized by abnormal cell proliferation comprising
administering to a subject in need thereof a selective DPP8/9 inhibitor and a fibroblast activation protein (FAP) inhibitor in an amount effective to inhibit the condition,
wherein the selective DPP8/9 inhibitor inhibits DPP8/9 to a greater extent than it inhibits at least DPP4.

6. The method of claim 5, further comprising administering a DPP4 inhibitor to the subject.

7. A method for improving the efficacy of anti-cancer treatment comprising administering to a subject in need thereof an anti-cancer agent and a selective DPP8/9 inhibitor in an amount effective to improve the effect of the anti-cancer agent,

5 wherein the selective DPP8/9 inhibitor inhibits DPP8/9 to a greater extent than it inhibits at least DPP4.

8. The method of claim 7, wherein the anti-cancer agent is a chemotherapeutic agent.

10 9. The method of claim 8, wherein the chemotherapeutic agent is docetaxel (TAXOTERE), cisplatin, gemcitabine, pemetrexed (ALIMTA), erlotinib (TARCEVA), gefitinib (IRESSA), temozolomide (TEMODAR), carboplatin, cyclophosphamide or doxorubicin.

15 10. The method of claim 7, wherein the anti-cancer agent is an immunotherapeutic agent.

11. The method of claim 10, wherein the immunotherapeutic agent is an antibody.

12. The method of claim 11, wherein the antibody is rituximab (RITUXAN), bevacizumab

20 (AVASTIN), cetuximab (ERBITUX), trastuzumab (HERCEPTIN), tositumomab (BEXXAR); or alemtuzumab (CAMPATH).

13. The method of claim 7, wherein the subject is refractory to a prior anti-cancer treatment.

25

14. The method of claim 13, wherein the prior anti-cancer treatment is different from the anti-cancer agent.

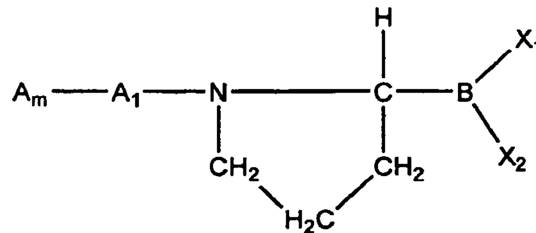
15. The method of claim 13, wherein the prior anti-cancer treatment comprises a

30 chemotherapeutic agent.

16. The method of claim 13 or 15, wherein the prior anti-cancer treatment comprises an immunotherapeutic agent.

17. A method for treating a subject having or at risk of developing a condition characterized by abnormal cell proliferation comprising

administering to a subject in need thereof a DPP8/9 inhibitor in an amount effective to
5 inhibit the condition, wherein the inhibitor is not



wherein m is an integer between 0 and 10, inclusive; A and A_1 may be L- or D-amino acid residues such that each A in A_m (i.e., where m>1) may be a different amino acid residue from
10 every other A in A_m; the C bonded to B is in the L-configuration; and each X_1 and X_2 is, independently, a hydroxyl group or a group capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH.

18. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is
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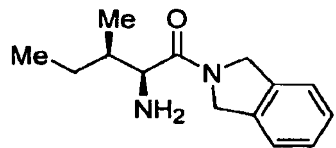

19. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is
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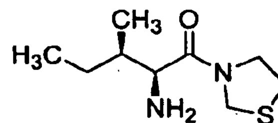
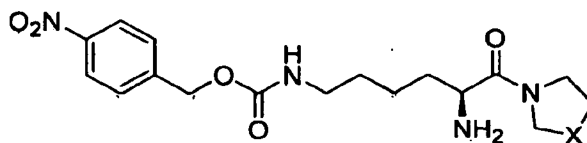


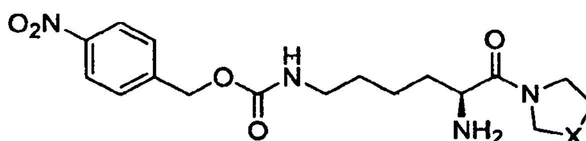
L-allo-isoleucyl thiazolidide .

20. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is



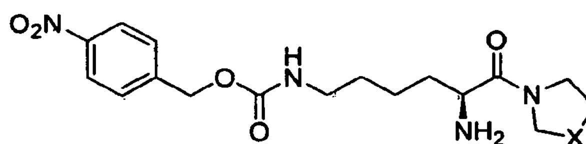
5 Lys[Z(NO_2)]-pyrrolidide (**8**, X = CH_2).

21. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is



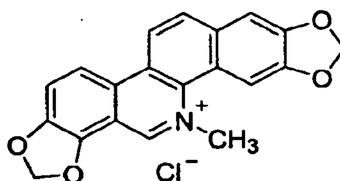
10 Lys[Z(NO_2)]-thiazolidide (**9**, X = S).

22. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is



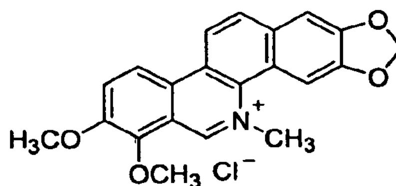
15 Lys[Z(NO_2)]-piperidide (**10**, X = CH_2CH_2).

23. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is



20 sanguinarine .

25 24. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is



chelerythrine .

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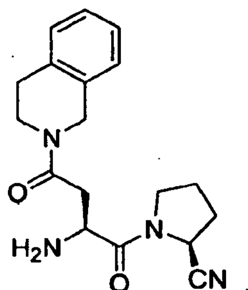
25. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is



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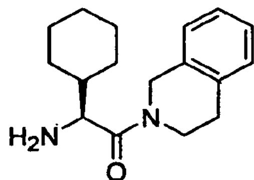
26. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is



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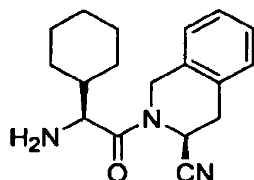
27. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is



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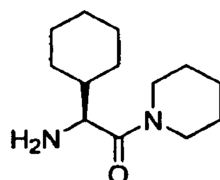
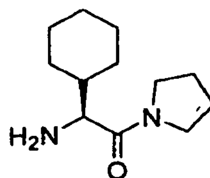
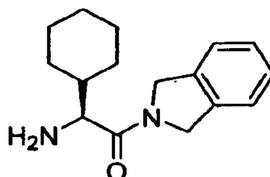
28. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is



20 29. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is



30. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is



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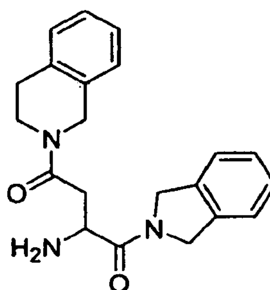
31. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is



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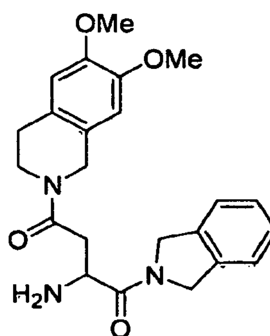
10 32. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is



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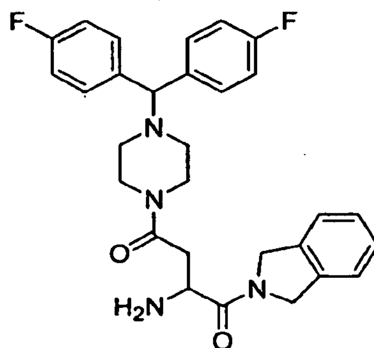
15 33. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is



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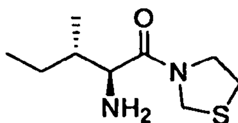
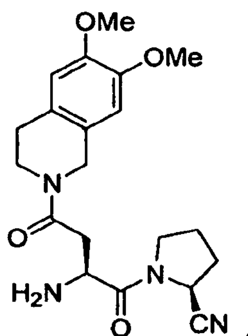
34. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is



5 35. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is



36. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is

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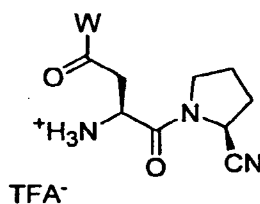

37. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is

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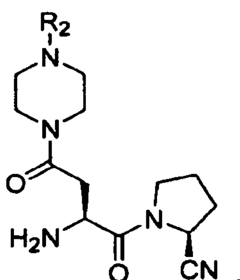
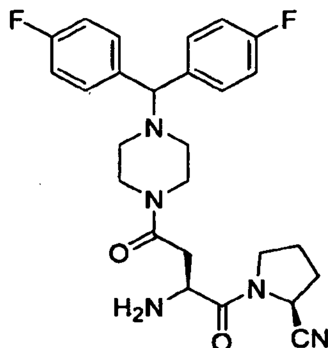
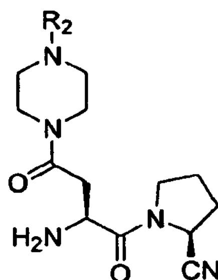


38. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is



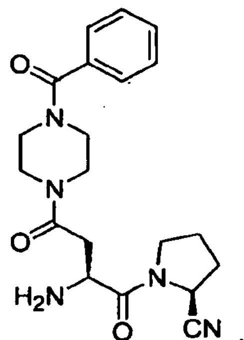
39. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is

5



wherein R_2 is nicotinonitrile.

10 40. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is



41. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is Arg-Pro-Ala-Tyr (SEQ ID NO: 1).

15

42. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is Met-Phe-Ala-Tyr (SEQ ID NO: 2).

43. The method of claim 1, 2, 3, 5, 7 or 17, wherein the selective DPP8/9 inhibitor is Ile-Phe-Ala-Tyr (SEQ ID NO: 3).

44. The method of claim 1, 2, 3, 5, 7 or 17, wherein the subject has a condition
5 characterized by abnormal cell proliferation.

45. The method of claim 1, 2, 3, 5, 7 or 17, wherein the subject is at risk of developing a condition characterized by abnormal cell proliferation.

10 46. The method of claim 1, 2, 3, 5 or 17, wherein the condition is cancer.

47. The method of claim 1, 2, 3, 5, 7 or 17, wherein the condition or the cancer is carcinoma.

15 48. The method of claim 1, 2, 3, 5, 7 or 17, wherein the condition or the cancer is non-small cell lung cancer.

49. The method of claim 1, 2, 3, 5, 7 or 17, wherein the condition or the cancer is pancreatic cancer.

20

50. The method of claim 1, 2, 3, 5, 7 or 17, wherein the condition or the cancer is colorectal cancer.

51. The method of claim 1, 2, 3, 5, 7 or 17, wherein the condition or the cancer is
25 melanoma.

52. The method of claim 1, 2, 3, 5, 7 or 17, wherein the condition or the cancer is sarcoma.

30 53. The method of claim 52, wherein the sarcoma is osteosarcoma.

54. The method of claim 52, wherein the sarcoma is fibrosarcoma.

55. The method of claim 1, 2, 3, 5, 7 or 17, wherein the condition or the cancer is leukemia or lymphoma.

56. The method of claim 1, 2, 3, 5, 7 or 17, wherein the condition or the cancer is chronic
5 lymphocytic leukemia.

57. The method of claim 1, 2, 3, 5, 7 or 17, wherein the condition or the cancer is refractory to a prior treatment.

10 58. The method of claim 1, 2, 3, 5, 7 or 17, wherein the condition or the cancer is a primary tumor.

59. The method of claim 1, 2, 3, 5 or 17, further comprising administering to the subject a second therapeutic agent that is an anti-cancer agent.

15

60. The method of claim 59, wherein the anti-cancer agent is a chemotherapeutic agent.

61. The method of claim 60, wherein the anti-cancer agent is docetaxel, cisplatin, gemcitabine, pemetrexed (ALIMTA), erlotinib (TARCEVA), gefitinib (IRESSA),
20 temozolomide (TEMODAR), carboplatin, cyclophosphamide or doxorubicin.

62. The method of claim 1, 2, 3, 5 or 17, further comprising administering to the subject a second therapeutic agent that is an immunotherapeutic agent.

25 63. The method of claim 62, wherein the immunotherapeutic agent is an antibody.

64. The method of claim 63, wherein the antibody is rituximab (RITUXAN), bevacizumab (AVASTIN), cetuximab (ERBITUX), trastuzumab (HERCEPTIN), tositumomab (BEXXAR), or alemtuzumab (CAMPATH).

30

65. The method of claim 62, wherein the immunotherapeutic agent is an antigen.

0

66. The method of claim 1, 2, 3, 5 or 17, further comprising administering to the subject a second therapeutic agent that is a cytokine.

67. The method of claim 66, wherein the cytokine is G-CSF or GM-CSF.

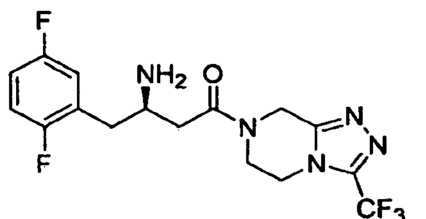
5

68. The method of claim 66, wherein the cytokine is M-CSF.

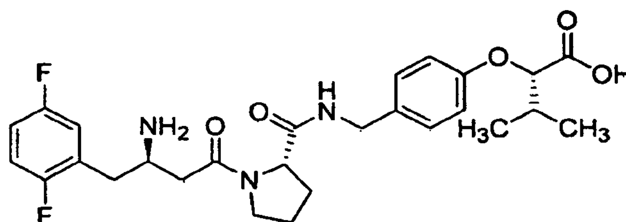
69. The method of claim 1, 2, 3, 5, 7 or 17, further comprising administering to the subject a DPP-IV inhibitor.

10

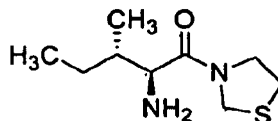
70. The method of claim 69, wherein the DPP4 inhibitor is



15 71. The method of claim 69, wherein the DPP4 inhibitor is



72. The method of claim 69, wherein the DPP4 inhibitor is



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L-*threo*-isoleucyl thiazolidide.

73. The method of claim 69, wherein the DPP4 inhibitor is

O=C1ONC(=N1)... (structure) NH3+ TFA- F

5-oxo-1,2,4-oxadiazole .

5 74. The method of claim 69, wherein the DPP4 inhibitor is

OH N H O N CN .

75. The method of claim 69, wherein the DPP4 inhibitor is

NC N N H H N O CN N .

10

76. The method of claim 69, wherein the DPP4 inhibitor is

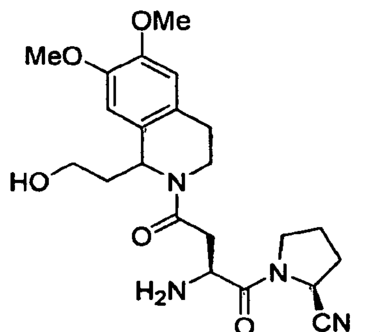
H_2N O N CN .

77. The method of claim 69, wherein the DPP4 inhibitor is

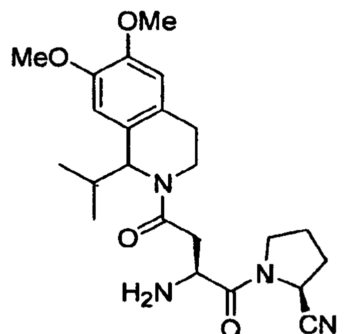
N O H_2N O N CN .

15

78. The method of claim 69 wherein the DPP4 inhibitor is



79. The method of claim 69, wherein the DPP4 inhibitor is



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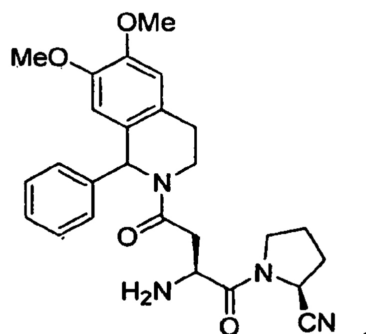
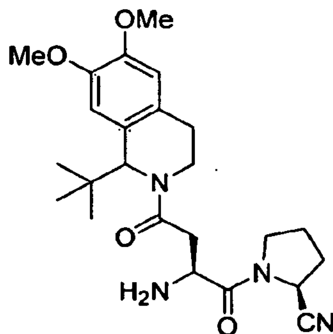
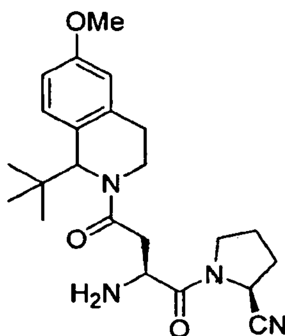
80. The method of claim 69, wherein the DPP4 inhibitor is



81. The method of claim 69, wherein the DPP4 inhibitor is



82. The method of claim 69, wherein the DPP4 inhibitor is



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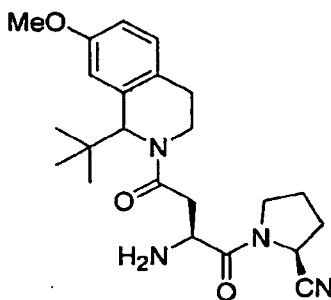
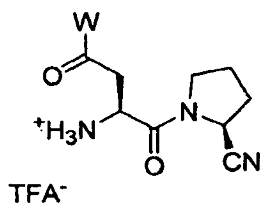
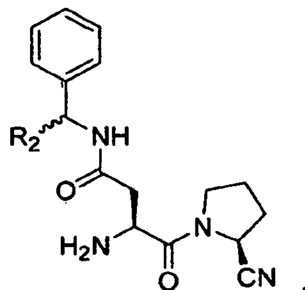
83. The method of claim 69, wherein the DPP4 inhibitor is



10 84. The method of claim 69, wherein the DPP4 inhibitor is

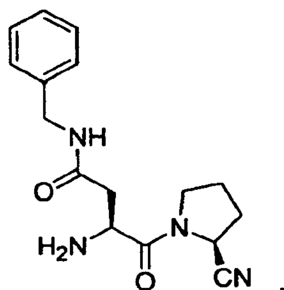


85. The method of claim 69, wherein the DPP4 inhibitor is

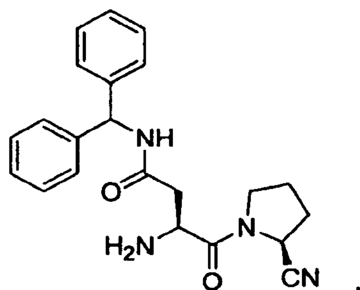


86. The method of claim 69, wherein the DPP4 inhibitor is

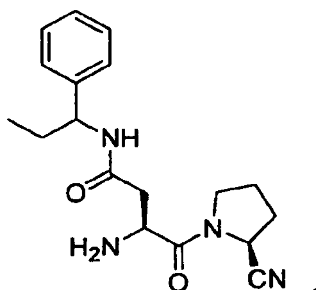
5



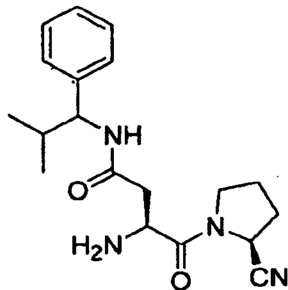
87. The method of claim 69, wherein the DPP4 inhibitor is



10 88. The method of claim 69, wherein the DPP4 inhibitor is

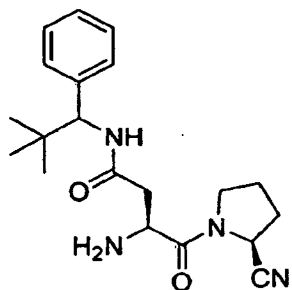


89. The method of claim 69, wherein the DPP4 inhibitor is

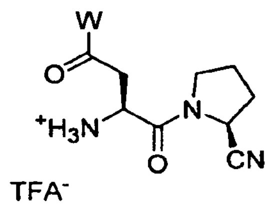


90. The method of claim 69, wherein the DPP4 inhibitor is

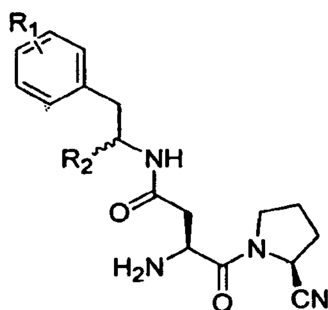
5



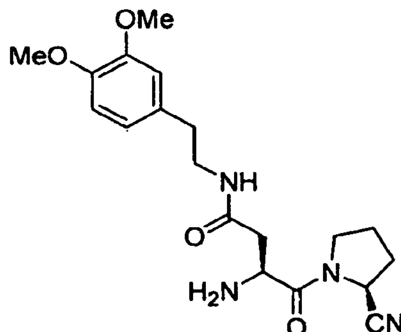
91. The method of claim 69, wherein the DPP4 inhibitor is



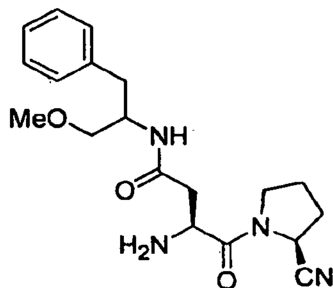
10 92. The method of claim 69, wherein the DPP4 inhibitor is



93. The method of claim 69, wherein the DPP4 inhibitor is

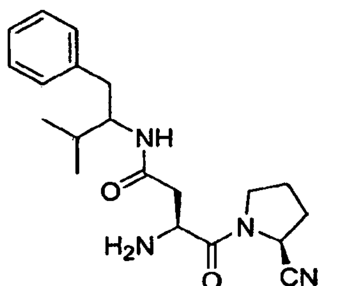


94. The method of claim 69, wherein the DPP4 inhibitor is



5

95. The method of claim 69, wherein the DPP4 inhibitor is



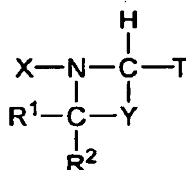
10 96. The method of claim 69, wherein the DPP4 inhibitor has an IC_{50} for DPP8/9 that is at least 3 fold higher than its IC_{50} for DPP4.

97. The method of claim 69, wherein the DPP4 inhibitor has an IC_{50} for DPP8/9 that is at least 5 fold higher than its IC_{50} for DPP4.

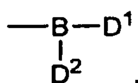
15

98. The method of claim 69, wherein the selective DPP8/9 inhibitor is administered prior to the DPP4 inhibitor.

99. The method of claim 1, 2, 3, 5, 7 or 17, further comprising administering to the subject a boroProline compound having a structure of

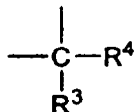


5 where T is a boronate group of the formula



where each D^1 and D^2, independently, is a hydroxyl group or a group which is capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH; X comprises an amino acid or a peptide which mimics the site of a substrate recognized by a post-prolyl

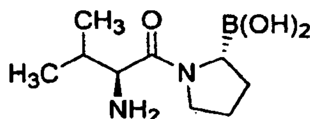
10 cleaving enzyme; Y is



and each R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8 is a hydrogen.

100. The method of claim 99, wherein the compound is

15



Val-boro-Pro (7)

101. The method of claim 99, wherein the selective DPP8/9 inhibitor is administered prior to the boroProline compound.

20

102. The method of claim 69, wherein the selective DPP8/9 inhibitor and the DPP4 inhibitor are administered to the subject substantially simultaneously.

103. The method of claim 69, wherein the selective DPP8/9 inhibitor and the DPP4

25 inhibitor are administered to the subject in an alternating manner.

104. The method of claim 99, wherein the selective DPP8/9 inhibitor and the boroProline compound are administered to the subject substantially simultaneously.

105. The method of claim 99, wherein the selective DPP8/9 inhibitor and the boroProline
5 compound are administered to the subject in an alternating manner.

106. The method of claim 1, wherein the subject has or is at risk of developing an infection.

107. The method of claim 106, wherein the infection is a viral infection.

10

108. The method of claim 106, further comprising administering to the subject an anti-microbial agent.

109. The method of claim 107, further comprising administering to the subject an anti-viral
15 agent.

110. The method of claim 46, wherein the cancer expresses normal or below-normal levels of DPP8/9 or does not express DPP8/9.

20

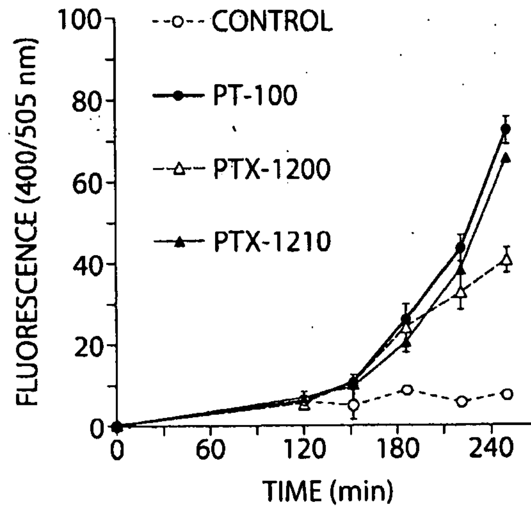


Fig. 1A

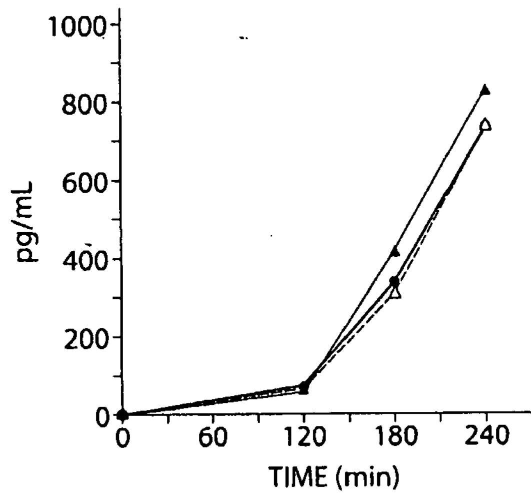


Fig. 1B

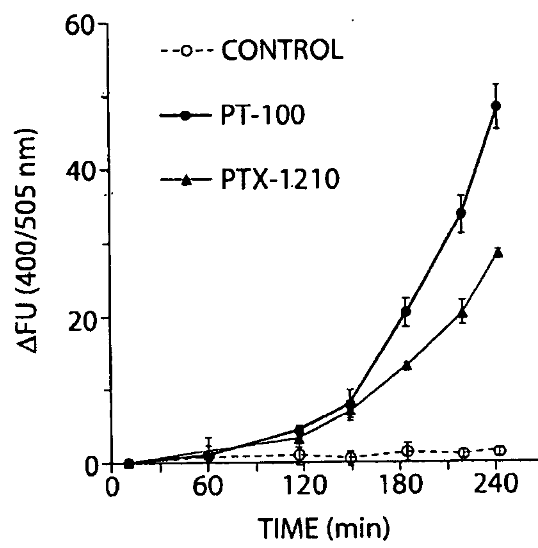


Fig. 2