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(54) Title: BIGUANIDE COMPOSITIONS AND METHODS RELATED THERETO

(57) Abstract: The present disclosure provides compositions comprising a biguanide compound and an immune therapy. Also provided are methods for treating cancer comprising conjoint administration of a biguanide compound and an immunotherapy.

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BIGUANIDE COMPOSITIONS AND METHODS RELATED THERETO

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with Government support under CA176337, awarded by the National Institutes of Health. The Government has certain rights in the invention.

REFERENCE TO RELATED APPLICATIONS

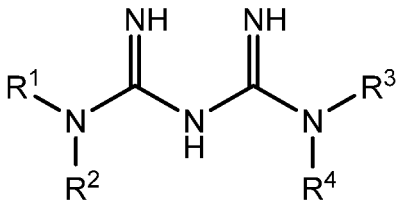
This application claims the benefit of U.S. Provisional Patent Application No. 62/592,148, filed November 29, 2017, the contents of which are fully incorporated by reference herein in their entirety.

BACKGROUND

Metformin analogues have shown some potential as cancer treatments. For example, U.S. Patent Pub. No. 2015/0158832, which is incorporated by reference herein in its entirety, provides a number of such analogues. Immune checkpoint inhibitors such as anti-PD1, anti-PD-L1, anti-CTLA-4 and anti-CD47 antibodies have also shown antitumor efficacy as single agents in some patients afflicted with melanoma, non-small cell lung cancer, renal cell carcinoma as well as bladder, gynecologic and breast cancers and a number of other malignancies. Extending the benefits of immunotherapies to greater numbers of patients afflicted with cancer is an important clinical goal going forward.

SUMMARY OF THE INVENTION

In certain aspects, the present disclosure provides compounds and compositions of formula I and pharmaceutically acceptable salts thereof:



(I)

wherein:

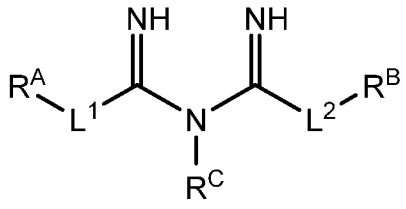
R^1 is H, alkyl or alkoxy;

R^2 is H, alkyl or alkoxy; or R^1 and R^2, taken together, complete a 5-10 member heterocycle;

R^3 is H, alkyl or alkoxy; and

R^4 is H, alkyl or alkoxy; or R^3 and R^4, taken together, complete a 5-10 member heterocycle.

In certain aspects, the present disclosure provides a pharmaceutical composition comprising a compound of formula A, or a pharmaceutically acceptable salt thereof, and an immune therapy:



(A)

wherein:

L^1 and L^2 are independently a bond or -NH-C(NH)-;

R^A is $-NR^1R^2$, alkyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl; R^1 and R^2 taken together with the N that separate them complete a heterocycle;

R^B is $-NR^3R^4$, alkyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl; or R^3 and R^4 taken together with the N that separate them complete a heterocycle;

R^1 is hydrogen, $-OR^5$, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl;

R^2 is hydrogen, $-OR^5$, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl; or ; R^1 and R^2 taken together with the N that separates them complete a heterocycle;

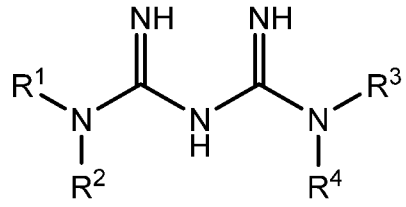
R^3 is hydrogen, $-OR^5$, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl;

R^4 is hydrogen, $-OR^5$, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl; or ; R^3 and R^4 taken together with the N that separates them complete a heterocycle;

R^C is hydrogen or C_1-C_5 alkyl; and

R^5 is hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl.

In certain embodiments, the compound of formula A is a compound of formula I:



(I).

In certain embodiments, R^1 is H, alkyl or alkoxy; R^2 is H, alkyl or alkoxy; or R^1 and R^2, taken together, complete a 5-10 member heterocycle; R^3 is H, alkyl or alkoxy; and R^4 is H, alkyl or alkoxy; or R^3 and R^4, taken together, complete a 5-10 member heterocycle.

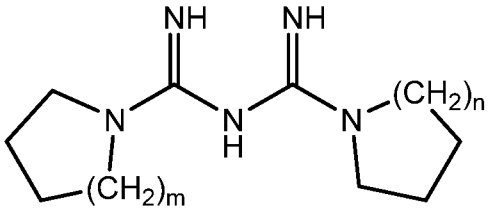
In certain embodiments, if R^1 and R^2 are methyl, then R^3 and R^4 are not both hydrogen.

In certain embodiments, R^1 is methoxy and R^2 is methyl.

In certain embodiments, R^1 is propyl and R^2 is methyl.

In certain embodiments, R^3 and R^4 are H.

In certain embodiments, the compound is of formula Ia, or a pharmaceutically acceptable salt thereof:



(Ia)

wherein:

m is 0, 1, 2, or 3; and

n is 0, 1, 2, or 3.

In certain embodiments, m and n are both 1.

In certain embodiments, the immune therapy is an immune checkpoint inhibitor, an indoleamine 2,3-dioxygenase inhibitor, or an adoptive cell transfer therapy.

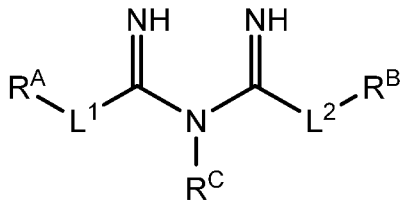
In certain embodiments, the immune therapy is an immune checkpoint inhibitor selected from anti-PD-1, anti-PD-L1, or anti-CTLA-4.

In certain embodiments, the immune checkpoint inhibitor is nivolumab, pembrolizumab, atezolizumab, durvalumab, avelumab, or ipilumumab.

In certain aspects, the present disclosure provides methods of treating cancer, comprising administering a compound or composition of formula I to a patient in need thereof.

In certain embodiments, the present disclosure is directed to a method for treating a cancer, comprising administering a composition as described herein to a patient in need thereof.

In certain embodiments, the present disclosure is directed to a method for treating a cancer, comprising administering conjointly a compound of formula A, or a pharmaceutically acceptable salt thereof, and an immune therapy:



(A)

wherein:

L^1 and L^2 are independently a bond or -NH-C(NH)-;

R^A is $-NR^1R^2$, alkyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl; R^1 and R^2 taken together with the N that separate them complete a heterocycle;

R^B is $-NR^3R^4$, alkyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl; or R^3 and R^4 taken together with the N that separate them complete a heterocycle;

R^1 is hydrogen, $-OR^5$, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl;

R^2 is hydrogen, $-OR^5$, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl; or ; R^1 and R^2 taken together with the N that separates them complete a heterocycle;

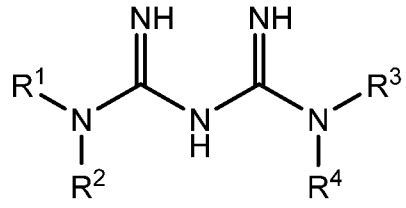
R^3 is hydrogen, $-OR^5$, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl;

R^4 is hydrogen, $-OR^5$, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl; or ; R^3 and R^4 taken together with the N that separates them complete a heterocycle;

R^C is hydrogen or C_1-C_5 alkyl; and

R^5 is hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl.

In certain embodiments, the compound of formula A is a compound of formula I:



(I).

In certain embodiments, R^1 is H, alkyl or alkoxy; R^2 is H, alkyl or alkoxy; or R^1 and R^2, taken together, complete a 5-10 member heterocycle; R^3 is H, alkyl or alkoxy; and R^4 is H, alkyl or alkoxy; or R^3 and R^4, taken together, complete a 5-10 member heterocycle.

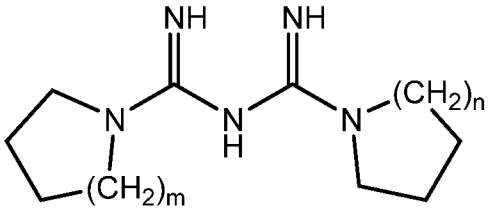
In certain embodiments, if R^1 and R^2 are methyl, then R^3 and R^4 are not both hydrogen.

In certain embodiments, R^1 is methoxy and R^2 is methyl.

In certain embodiments, R^1 is propyl and R^2 is methyl.

In certain embodiments, R^3 and R^4 are H.

In certain embodiments, the compound is of formula Ia, or a pharmaceutically acceptable salt thereof:



(Ia)

wherein:

m is 0, 1, 2, or 3; and

n is 0, 1, 2, or 3.

In certain embodiments, m and n are both 1.

In certain embodiments, the immune therapy is an immune checkpoint inhibitor, an indoleamine 2,3-dioxygenase inhibitor, or an adoptive cell transfer therapy.

In certain embodiments, the immune therapy is an immune checkpoint inhibitor selected from anti-PD-1, anti-PD-L1, anti-CTLA-4 or anti-CD47.

In certain embodiments, the immune checkpoint inhibitor is nivolumab, pembrolizumab, atezolizumab, durvalumab, avelumab, or ipilumumab.

In certain embodiments, the cancer is leukemia, lymphoma, Hodgkins lymphoma, colon cancer, breast cancer, prostate cancer, lung cancer, skin cancer, liver cancer, pancreatic cancer, ovarian cancer, bladder cancer, kidney cancer, bile duct cancer, esophageal cancer, cervical cancer, endometrial cancer, melanoma, head and neck cancer, brain cancer, glioma, neuroblastoma, osteosarcoma, chondrosarcoma, gastric carcinoma, glioma, mesothelioma, Kaposi sarcoma, liposarcoma, synovial sarcoma, or Wilm's tumor.

In certain embodiments, the cancer is melanoma, non-small cell lung cancer, renal cell carcinoma, bladder cancer, triple-negative breast cancer, or a gynecologic cancer such as endometrial, cervical, or ovarian cancer.

In certain embodiments, the cancer is breast cancer, such as triple-negative breast cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A shows inhibition of human MDA-MB-231 tumor xenograft progression by metformin analogue 005. TNBC subcutaneous xenografts are used with 5 mice/group. Metformin (250 mg/kg), analogue 005 (50 mg/kg) or control were given Qd by oral gavage

after tumors were 50-75 mm^3. Antitumor effects of analogue 005 exceeded those of metformin or controls ($P<0.001$). Of note, mouse body weights were not significantly different from controls in the analogue group, suggesting little toxicity *in vivo*.

FIG. 1B shows suppression of 4T1 TNBC xenograft progression *in vivo* in syngeneic, immune-competent BALB/c mouse models. BALB/c female mice (6-7 wk old) were injected in the flanks with 1 x 10^5 4T1 cells s.c. using established methods. Mice were randomized when tumor volumes were 200-250 mm^3. For treatment, mice were divided into groups: (a) vehicle control (CON), (b) metformin analogue 006 (50 mg/kg/day by oral gavage) (006), (c) anti-PD-L1 antibody (Biolegend anti-CD274/B7-H1/PD-L1 clone 10F.9G2, 100 μg/mouse IP diluted in PBS, Q3 days) (PD-L1) and (d) dual treatment with both 006 and anti-PD-L1 (006 + PD-L1). Tumor sizes were recorded every 2-3 days; mice were weighed twice weekly. Analogue 006 alone or with anti-PD-L1 suppresses TNBC progression *in vivo* versus controls (*$P <0.001$, t-test). Studies used 5 mice/group. Of note, dual treatment with 006 + anti-PD-L1 also shows a significantly greater effect at suppressing 4T1 TNBC growth than that of anti-PD-L1 alone. .

FIG 2A shows the effects of biguanide 006 on CD8+ tumor-infiltrating lymphocyte (TIL) populations *in vivo*. Mice were sacrificed at day 10 post-treatment (N=3), with tumor cell populations processed and analyzed by cyTOF. **FIG. 2A**. Fold change in CD8+ T-cell infiltration in tumors after treatment with metformin analogue 006 relative to the vehicle-treated control group.

FIGs. 2B and 2C show increased expression of biomarkers for CD8+-TIL population activation. Single cell suspensions were isolated from 4T1 tumors grown in BALB/c mice and stained with different antibodies against cell surface, nuclear and secreted proteins. After staining, cell suspensions were fixed, processed and subjected to mass cytometry. Figure panels show CD8+ T-cell expression of different activation markers following treatments: **FIG 2B**: Interferon gamma (IFNγ), **FIG. 2C**: proliferation marker Ki-67.

FIGs. 3A and 3B show that combination therapy with biguanide 006 and anti-PD-L1 antibody results in an apparent decrease in the expansion of G-MDSCs derived from tumors of BALB/c mice with 4T1 breast tumor implants. **FIG. 3A** shows a graphic representation of a viSNE clustering analysis using Cytobank that defines populations of distinct immune cells from tumors and spleens. Equal event sampling using 10,000 events per individual sample was used. Cellular phenotypes were assigned to the viSNE plot based on distribution and expression characteristics using phenotypic markers: B cells ($CD19^+CD220^+$), $CD3^+CD4^+$

and $CD3^+CD8^+$ TILs, Dendritic Cells (DC, $CD11c^+MHCII^+$), Natural Killer cells (NK, $CD335^+CD161^+$), G-MDSC ($CD11b^+Ly6G^{hi}Ly6C^{low}$) M-MDSC ($CD11b^+Ly6C^{hi}Ly6G^{low}$). Expression of G-MDSC (CD11b+ Ly6G+) in the gated CD45+ cells from tumors were measured by mass cytometry. Representative plots are shown in **FIG. 3B**, N=5.

FIG. 3C shows a mass cytometry gating strategy using a Helios cyTOF platform (representative example). The sequential gating strategy for analysis of spleen and tumor cell subsets is shown. Dead cell events and CyTOF calibration beads were excluded before gating on CD45+ leukocytes. Single cell events were identified using Iridium-Intercalator according to manufacturer's protocol. Distinct immune cell subsets were further analyzed for differences in functional marker expression, CD3+CD4 and CD3+CD8+ lymphocytes, granulocytic myeloid suppressor cells (G-MDSC, $CD11b+Ly6G^{hi}$ $Ly6C^{low}$), monocytic myeloid suppressor cells (M-MDSC CD11b+ $Ly6C^{hi}Ly6G^{low}$), regulatory dendritic cells (rDCs - CD11c+CD11c-MHCII+), myeloid dendritic cells (mDCs - CD11c+ MHCII+), macrophages (Macro, CD11b+, F4/80+) and Tregs among others (CD4+CD25+ FoxP3+). Panel shows cyTOF data from a spleen sample experiment. Plots include concatenated data from one experiment (n = 5 mice).

FIG. 4 shows that biguanide 006 inhibits expansion of human myeloid derived suppressor cells (MDSC) derived from bone marrow of breast cancer patients. Bone marrow cells were incubated in the presence of GM-CSF and IL-6 for 6 days in RPMI medium + 15% FBS in the presence of vehicle or 1 mM biguanide 006. After 6 days cells were subjected to flow cytometry as described before (1,2). The graph shows the total number of MDSC (CD45+CD3-Cd19-CD19-CD20-Cd56-) after expansion as described before (see 14- -21).

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure provides compositions of metformin and analogues with immune checkpoint inhibitors, and related methods of treatment. Metformin analogues enhance the therapeutic benefit of immune checkpoint inhibitors in triple-negative breast cancer as well as in other malignancies. Metformin analogues block the expansion and activation of myeloid-derived suppressor cells that would otherwise allow tumors to escape immune surveillance and attack. In addition, metformin analogues impact the trafficking and activity of other immune cells such as CD8-positive T-cells and potentially subsets of tumor-associated macrophages and dendritic cell subpopulations.. The antitumor effects elicited by these new metformin analogues and their interactions with immune checkpoint inhibitors are

expected to boost antitumor activity of immune checkpoint inhibitors and address an unmet clinical need.

Metformin belongs to the biguanide class of pharmaceuticals and exhibits modest activity as a single agent against triple-negative breast cancer (TNBC) in preclinical and early clinical reports (1-3). Treatment of diabetic patients with metformin, but not other antidiabetes drugs, is associated with a 30-40% reduction in the incidence of cancer (4-6); yet the underlying mechanism of these outcomes is poorly understood. Emerging evidence suggests that the reported antitumor activity of metformin may be in part immune-mediated (7).

Both anti-PD-1 and anti-PD-L1 treatments, as well as a number of other immune checkpoint inhibitors, show promising responses for a fraction of triple-negative breast cancer (TNBC) patients, yet significant progress is urgently needed to achieve objective responses in the majority of those diagnosed with TNBC (8, 9). Most patients do not derive benefits from anti-PD-L1 treatment alone and a combination therapy is needed to provide synergism and enhance response rates to current immune-based therapies.

The compositions and methods of the present disclosure seek to more effectively harness the immune response and, ultimately, provide a new treatment option for TNBC patients. The metformin analogues shown in the following scheme were prepared and their IC_{50}s determined based on *in vitro* assays of MDA-MB-231 human TNBC cell proliferation over a range of doses using established methods (27, 28).

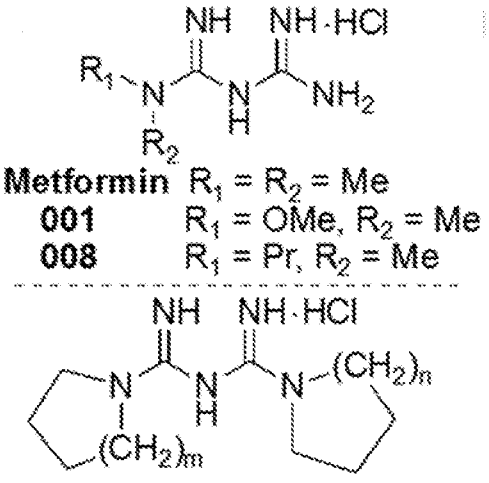


Table 1: IC_{50} against MDA-MB-231

Compound	IC_{50} (μM)
Metformin	>5000
001	2821
002	450
005	260
006	230
008	342

The observed antitumor effects of these biguanides may be a result of direct AMPK-cell signaling modulation in addition to indirect immunomodulatory effects via trafficking of $CD8^+$ TILs and inhibition of MDSC expansion and activity.

Pharmaceutical Compositions

The compositions and methods of the present invention may be utilized to treat an individual in need thereof. In certain embodiments, the individual is a mammal such as a human, or a non-human mammal. When administered to an animal, such as a human, the composition or the compound is preferably administered as a pharmaceutical composition comprising, for example, a compound of the invention and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are well known in the art and include, for example, aqueous solutions such as water or physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil, or injectable organic esters. In preferred embodiments, when such pharmaceutical compositions are for human administration, particularly for invasive routes of administration (i.e., routes, such as injection or implantation, that circumvent transport or diffusion through an epithelial barrier), the aqueous solution is pyrogen-free, or substantially pyrogen-free. The excipients can be chosen, for example, to effect delayed release of an agent or to selectively target one or more cells, tissues or organs. The pharmaceutical composition can be in dosage unit form such as tablet, capsule (including sprinkle capsule and gelatin capsule), granule, lyophile for reconstitution, powder, solution, syrup, suppository, injection or the like. The composition can also be present in a transdermal delivery system, e.g., a skin patch. The composition can

also be present in a solution suitable for topical administration, such as a lotion, cream, or ointment.

A pharmaceutically acceptable carrier can contain physiologically acceptable agents that act, for example, to stabilize, increase solubility or to increase the absorption of a compound such as a compound of the invention. Such physiologically acceptable agents include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other stabilizers or excipients. The choice of a pharmaceutically acceptable carrier, including a physiologically acceptable agent, depends, for example, on the route of administration of the composition. The preparation or pharmaceutical composition can be a selfemulsifying drug delivery system or a selfmicroemulsifying drug delivery system. The pharmaceutical composition (preparation) also can be a liposome or other polymer matrix, which can have incorporated therein, for example, a compound of the invention. Liposomes, for example, which comprise phospholipids or other lipids, are nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free

water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

A pharmaceutical composition (preparation) can be administered to a subject by any of a number of routes of administration including, for example, orally (for example, drenches as in aqueous or non-aqueous solutions or suspensions, tablets, capsules (including sprinkle capsules and gelatin capsules), boluses, powders, granules, pastes for application to the tongue); absorption through the oral mucosa (e.g., sublingually); subcutaneously; transdermally (for example as a patch applied to the skin); and topically (for example, as a cream, ointment or spray applied to the skin). The compound may also be formulated for inhalation. In certain embodiments, a compound may be simply dissolved or suspended in sterile water. Details of appropriate routes of administration and compositions suitable for same can be found in, for example, U.S. Pat. Nos. 6,110,973, 5,763,493, 5,731,000, 5,541,231, 5,427,798, 5,358,970 and 4,172,896, as well as in patents cited therein.

The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient that can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 1 percent to about ninety-nine percent of active ingredient, preferably from about 5 percent to about 70 percent, most preferably from about 10 percent to about 30 percent.

Methods of preparing these formulations or compositions include the step of bringing into association an active compound, such as a compound of the invention, with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules (including sprinkle capsules and gelatin capsules), cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), lyophile, powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-

water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. Compositions or compounds may also be administered as a bolus, electuary or paste.

To prepare solid dosage forms for oral administration (capsules (including sprinkle capsules and gelatin capsules), tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; (10) complexing agents, such as, modified and unmodified cyclodextrins; and (11) coloring agents. In the case of capsules (including sprinkle capsules and gelatin capsules), tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions, such as dragees, capsules (including sprinkle capsules and gelatin capsules), pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so

as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions that can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms useful for oral administration include pharmaceutically acceptable emulsions, lyophiles for reconstitution, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, cyclodextrins and derivatives thereof, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Dosage forms for the topical or transdermal administration include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants that may be required.

The ointments, pastes, creams and gels may contain, in addition to an active compound, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch,

tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to an active compound, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the active compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the compound in a polymer matrix or gel.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intraocular (such as intravitreal), intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion. Pharmaceutical compositions suitable for parenteral administration comprise one or more active compounds in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents that delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution, which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsulated matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions that are compatible with body tissue.

For use in the methods of this invention, active compounds can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

Methods of introduction may also be provided by rechargeable or biodegradable devices. Various slow release polymeric devices have been developed and tested *in vivo* in recent years for the controlled delivery of drugs, including proteinaceous biopharmaceuticals. A variety of biocompatible polymers (including hydrogels), including both biodegradable and non-degradable polymers, can be used to form an implant for the sustained release of a compound at a particular target site.

Actual dosage levels of the active ingredients in the pharmaceutical compositions may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular compound or combination of compounds employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound(s) being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound(s) employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the therapeutically effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the pharmaceutical composition or compound at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. By "therapeutically effective amount" is meant the concentration of a compound that is sufficient to elicit the desired therapeutic effect. It is generally understood that the effective amount of the compound will vary according to the weight, sex, age, and medical history of the subject. Other factors which influence the effective amount may include, but are not limited to, the severity of the patient's condition, the disorder being treated, the stability of the compound, and, if desired, another type of therapeutic agent being administered with the compound of the invention. A larger total dose can be delivered by multiple administrations of the agent. Methods to determine efficacy and dosage are known to those skilled in the art (Isselbacher et al. (1996) Harrison's Principles of Internal Medicine 13 ed., 1814-1882, herein incorporated by reference).

In general, a suitable daily dose of an active compound used in the compositions and methods of the invention will be that amount of the compound that is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above.

If desired, the effective daily dose of the active compound may be administered as one, two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. In certain embodiments of the present invention, the active compound may be administered two or three times daily. In preferred embodiments, the active compound will be administered once daily.

The patient receiving this treatment is any animal in need, including primates, in particular humans; and other mammals such as equines, cattle, swine, sheep, cats, and dogs; poultry; and pets in general.

In certain embodiments, compounds of the invention may be used alone or conjointly administered with another type of therapeutic agent.

The present disclosure includes the use of pharmaceutically acceptable salts of compounds of the invention in the compositions and methods of the present invention. In certain embodiments, contemplated salts of the invention include, but are not limited to, alkyl, dialkyl, trialkyl or tetra-alkyl ammonium salts. In certain embodiments, contemplated salts of the invention include, but are not limited to, L-arginine, benenthamine, benzathine, betaine, calcium hydroxide, choline, deanol, diethanolamine, diethylamine, 2-(diethylamino)ethanol, ethanolamine, ethylenediamine, N-methylglucamine, hydrabamine, 1H-imidazole, lithium, L-lysine, magnesium, 4-(2-hydroxyethyl)morpholine, piperazine, potassium, 1-(2-hydroxyethyl)pyrrolidine, sodium, triethanolamine, tromethamine, and zinc salts. In certain embodiments, contemplated salts of the invention include, but are not limited to, Na, Ca, K, Mg, Zn or other metal salts. In certain embodiments, contemplated salts of the invention include, but are not limited to, 1-hydroxy-2-naphthoic acid, 2,2-dichloroacetic acid, 2-hydroxyethanesulfonic acid, 2-oxoglutaric acid, 4-acetamidobenzoic acid, 4-aminosalicylic acid, acetic acid, adipic acid, l-ascorbic acid, l-aspartic acid, benzenesulfonic acid, benzoic acid, (+)-camphoric acid, (+)-camphor-10-sulfonic acid, capric acid (decanoic acid), caproic acid (hexanoic acid), caprylic acid (octanoic acid), carbonic acid, cinnamic acid, citric acid, cyclamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, d-glucoheptonic acid, d-gluconic acid, d-glucuronic acid, glutamic acid, glutaric acid, glycerophosphoric acid, glycolic acid, hippuric acid, hydrobromic acid, hydrochloric acid, isobutyric acid, lactic acid, lactobionic acid, lauric acid, maleic acid, l-malic acid, malonic acid, mandelic acid, methanesulfonic acid , naphthalene-1,5-disulfonic acid, naphthalene-2-sulfonic acid, nicotinic acid, nitric acid, oleic acid, oxalic acid, palmitic acid, pamoic acid, phosphoric acid, proprionic acid, l-pyroglutamic acid, salicylic acid, sebacic acid, stearic acid, succinic acid, sulfuric acid, l-tartaric acid, thiocyanic acid, p-toluenesulfonic acid, trifluoroacetic acid, and undecylenic acid acid salts.

The pharmaceutically acceptable acid addition salts can also exist as various solvates, such as with water, methanol, ethanol, dimethylformamide, and the like. Mixtures of such

solvates can also be prepared. The source of such solvate can be from the solvent of crystallization, inherent in the solvent of preparation or crystallization, or adventitious to such solvent.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically acceptable antioxidants include: (1) water-soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal-chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Definitions

Unless otherwise defined herein, scientific and technical terms used in this application shall have the meanings that are commonly understood by those of ordinary skill in the art. Generally, nomenclature used in connection with, and techniques of, chemistry, cell and tissue culture, molecular biology, cell and cancer biology, neurobiology, neurochemistry, virology, immunology, microbiology, pharmacology, genetics and protein and nucleic acid chemistry, described herein, are those well known and commonly used in the art.

The methods and techniques of the present disclosure are generally performed, unless otherwise indicated, according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout this specification. See, e.g. “Principles of Neural Science”, McGraw-Hill Medical, New York, N.Y. (2000); Motulsky, “Intuitive Biostatistics”, Oxford University Press, Inc. (1995); Lodish et al., “Molecular Cell Biology, 4th ed.”, W. H. Freeman & Co., New York (2000); Griffiths et al., “Introduction to Genetic Analysis, 7th ed.”, W. H. Freeman & Co., N.Y. (1999); and Gilbert et al., “Developmental Biology, 6th ed.”, Sinauer Associates, Inc., Sunderland, MA (2000).

Chemistry terms used herein, unless otherwise defined herein, are used according to conventional usage in the art, as exemplified by "The McGraw-Hill Dictionary of Chemical Terms", Parker S., Ed., McGraw-Hill, San Francisco, C.A. (1985).

All of the above, and any other publications, patents and published patent applications referred to in this application are specifically incorporated by reference herein. In case of conflict, the present specification, including its specific definitions, will control.

The term "agent" is used herein to denote a chemical compound (such as an organic or inorganic compound, a mixture of chemical compounds), a biological macromolecule (such as a nucleic acid, an antibody, including parts thereof as well as humanized, chimeric and human antibodies and monoclonal antibodies, a protein or portion thereof, e.g., a peptide, a lipid, a carbohydrate), or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian) cells or tissues. Agents include, for example, agents whose structure is known, and those whose structure is not known.

A "patient," "subject," or "individual" are used interchangeably and refer to either a human or a non-human animal. These terms include mammals, such as humans, primates, livestock animals (including bovines, porcines, etc.), companion animals (e.g., canines, felines, etc.) and rodents (e.g., mice and rats).

"Treating" a condition or patient refers to taking steps to obtain beneficial or desired results, including clinical results. As used herein, and as well understood in the art, "treatment" is an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment.

The term "preventing" is art-recognized, and when used in relation to a condition, such as a local recurrence (e.g., pain), a disease such as cancer, a syndrome complex such as heart failure or any other medical condition, is well understood in the art, and includes administration of a composition which reduces the frequency of, or delays the onset of, symptoms of a medical condition in a subject relative to a subject which does not receive the composition. Thus, prevention of cancer includes, for example, reducing the number of detectable cancerous growths in a population of patients receiving a prophylactic treatment

relative to an untreated control population, and/or delaying the appearance of detectable cancerous growths in a treated population versus an untreated control population, e.g., by a statistically and/or clinically significant amount.

"Administering" or "administration of" a substance, a compound or an agent to a subject can be carried out using one of a variety of methods known to those skilled in the art. For example, a compound or an agent can be administered, intravenously, arterially, intradermally, intramuscularly, intraperitoneally, subcutaneously, ocularly, sublingually, orally (by ingestion), intranasally (by inhalation), intraspinally, intracerebrally, and transdermally (by absorption, e.g., through a skin duct). A compound or agent can also appropriately be introduced by rechargeable or biodegradable polymeric devices or other devices, e.g., patches and pumps, or formulations, which provide for the extended, slow or controlled release of the compound or agent. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

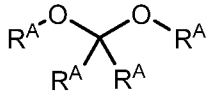
Appropriate methods of administering a substance, a compound or an agent to a subject will also depend, for example, on the age and/or the physical condition of the subject and the chemical and biological properties of the compound or agent (e.g., solubility, digestibility, bioavailability, stability and toxicity). In some embodiments, a compound or an agent is administered orally, e.g., to a subject by ingestion. In some embodiments, the orally administered compound or agent is in an extended release or slow release formulation, or administered using a device for such slow or extended release.

As used herein, the phrase "conjoint administration" refers to any form of administration of two or more different therapeutic agents such that the second agent is administered while the previously administered therapeutic agent is still effective in the body (e.g., the two agents are simultaneously effective in the patient, which may include synergistic effects of the two agents). For example, the different therapeutic compounds can be administered either in the same formulation or in separate formulations, either concomitantly or sequentially. Thus, an individual who receives such treatment can benefit from a combined effect of different therapeutic agents.

A "therapeutically effective amount" or a "therapeutically effective dose" of a drug or agent is an amount of a drug or an agent that, when administered to a subject will have the intended therapeutic effect. The full therapeutic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a therapeutically effective amount may be administered in one or more administrations.

The precise effective amount needed for a subject will depend upon, for example, the subject's size, health and age, and the nature and extent of the condition being treated, such as cancer or MDS. The skilled worker can readily determine the effective amount for a given situation by routine experimentation.

The term "acetal" is art-recognized and may be represented by the general formula



wherein each R^A independently represents hydrogen or a hydrocarbyl, such as alkyl, or any occurrence of R^A taken together with another and the intervening atom(s) complete a carbocycle or heterocycle having from 4 to 8 atoms in the ring structure.

The term "acyl" is art-recognized and refers to a group represented by the general formula hydrocarbylC(O)-, preferably alkylC(O)-.

The term "acylamino" is art-recognized and refers to an amino group substituted with an acyl group and may be represented, for example, by the formula hydrocarbylC(O)NH-.

The term "acyloxy" is art-recognized and refers to a group represented by the general formula hydrocarbylC(O)O-, preferably alkylC(O)O-.

The term "alkoxy" refers to an alkyl group, preferably a lower alkyl group, having an oxygen attached thereto. Representative alkoxy groups include methoxy, trifluoromethoxy, ethoxy, propoxy, tert-butoxy and the like.

The term "alkoxyalkyl" refers to an alkyl group substituted with an alkoxy group and may be represented by the general formula alkyl-O-alkyl.

The term "alkenyl", as used herein, refers to an aliphatic group containing at least one double bond and is intended to include both "unsubstituted alkenyls" and "substituted alkenyls", the latter of which refers to alkenyl moieties having substituents replacing a hydrogen on one or more carbons of the alkenyl group. Such substituents may occur on one or more carbons that are included or not included in one or more double bonds. Moreover, such substituents include all those contemplated for alkyl groups, as discussed below, except where stability is prohibitive. For example, substitution of alkenyl groups by one or more alkyl, carbocyclyl, aryl, heterocyclyl, or heteroaryl groups is contemplated.

An "alkyl" group or "alkane" is a straight chained or branched non-aromatic hydrocarbon which is completely saturated. Typically, a straight chained or branched alkyl group has from 1 to about 20 carbon atoms, preferably from 1 to about 10 unless otherwise

defined. Examples of straight chained and branched alkyl groups include methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, tert-butyl, pentyl, hexyl, pentyl and octyl. A C_1-C_6 straight chained or branched alkyl group is also referred to as a "lower alkyl" group.

Moreover, the term "alkyl" (or "lower alkyl") as used throughout the specification, examples, and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents, if not otherwise specified, can include, for example, a halogen (e.g., fluoro), a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxy, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. In preferred embodiments, the substituents on substituted alkyls are selected from C_{1-6} alkyl, C_{3-6} cycloalkyl, halogen, carbonyl, cyano, or hydroxyl. In more preferred embodiments, the substituents on substituted alkyls are selected from fluoro, carbonyl, cyano, or hydroxyl. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. For instance, the substituents of a substituted alkyl may include substituted and unsubstituted forms of amino, azido, imino, amido, phosphoryl (including phosphonate and phosphinate), sulfonyl (including sulfate, sulfonamido, sulfamoyl and sulfonate), and silyl groups, as well as ethers, alkylthios, carbonyls (including ketones, aldehydes, carboxylates, and esters), $-CF_3$, -CN and the like. Exemplary substituted alkyls are described below. Cycloalkyls can be further substituted with alkyls, alkenyls, alkoxys, alkylthios, aminoalkyls, carbonyl-substituted alkyls, $-CF_3$, -CN, and the like.

The term "C_{x-y}" when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups that contain from x to y carbons in the chain. For example, the term "C_{x-y} alkyl" refers to substituted or unsubstituted saturated hydrocarbon groups, including straight-chain alkyl and branched-chain alkyl groups that contain from x to y carbons in the chain, including haloalkyl groups. Preferred haloalkyl groups include trifluoromethyl, difluoromethyl, 2,2,2-trifluoroethyl, and pentafluoroethyl. C_0 alkyl indicates a hydrogen where the group is in a terminal position, a bond if internal. The terms "C_{2-y} alkenyl" and "C_{2-y} alkynyl" refer to substituted or unsubstituted unsaturated

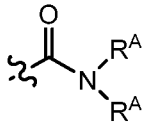
aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

The term “alkylamino”, as used herein, refers to an amino group substituted with at least one alkyl group.

The term “alkylthio”, as used herein, refers to a thiol group substituted with an alkyl group and may be represented by the general formula alkylS-.

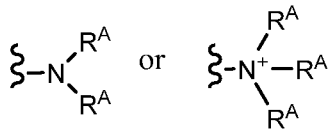
The term “alkynyl”, as used herein, refers to an aliphatic group containing at least one triple bond and is intended to include both "unsubstituted alkynyls" and "substituted alkynyls", the latter of which refers to alkynyl moieties having substituents replacing a hydrogen on one or more carbons of the alkynyl group. Such substituents may occur on one or more carbons that are included or not included in one or more triple bonds. Moreover, such substituents include all those contemplated for alkyl groups, as discussed above, except where stability is prohibitive. For example, substitution of alkynyl groups by one or more alkyl, carbocyclyl, aryl, heterocyclyl, or heteroaryl groups is contemplated.

The term “amide”, as used herein, refers to a group



wherein each R^A independently represent a hydrogen or hydrocarbyl group, or two R^A are taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

The terms “amine” and “amino” are art-recognized and refer to both unsubstituted and substituted amines and salts thereof, e.g., a moiety that can be represented by



wherein each R^A independently represents a hydrogen or a hydrocarbyl group, or two R^A are taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

The term “aminoalkyl”, as used herein, refers to an alkyl group substituted with an amino group.

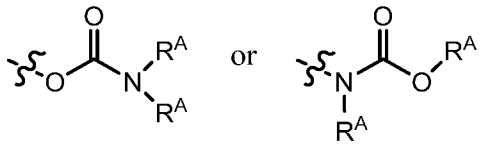
The term “aralkyl”, as used herein, refers to an alkyl group substituted with an aryl group.

The term "aryl" as used herein include substituted or unsubstituted single-ring aromatic groups in which each atom of the ring is carbon. Preferably the ring is a 6- or 10-membered ring, more preferably a 6-membered ring. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is aromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Aryl groups include benzene, naphthalene, phenanthrene, phenol, aniline, and the like.

The term "boron" as used herein with respect to a substituent on an organic compound, is art-recognized and refers to a group $-B(R^A)_2$, wherein each R^A independently represents hydrogen or a hydrocarbyl, such as alkyl, or any occurrence of R^A taken together with another and the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

The term "boronic ester" or "boronate ester" as used herein is art-recognized and refers to a group $-B(OR^A)_2$, wherein each R^A independently represents hydrogen or a hydrocarbyl, such as alkyl, or any occurrence of R^A taken together with another and the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

The term "carbamate" is art-recognized and refers to a group



wherein each R^A independently represent hydrogen or a hydrocarbyl group, such as an alkyl group, or both R^A taken together with the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

The terms "carbocycle", and "carbocyclic", as used herein, refers to a saturated or unsaturated ring in which each atom of the ring is carbon. The term carbocycle includes both aromatic carbocycles and non-aromatic carbocycles. Non-aromatic carbocycles include both cycloalkane rings, in which all carbon atoms are saturated, and cycloalkene rings, which contain at least one double bond. "Carbocycle" includes 5-7 membered monocyclic and 8-12 membered bicyclic rings. Each ring of a bicyclic carbocycle may be selected from saturated, unsaturated and aromatic rings. Carbocycle includes bicyclic molecules in which one, two or three or more atoms are shared between the two rings. The term "fused carbocycle" refers to

a bicyclic carbocycle in which each of the rings shares two adjacent atoms with the other ring. Each ring of a fused carbocycle may be selected from saturated, unsaturated and aromatic rings. In an exemplary embodiment, an aromatic ring, e.g., phenyl, may be fused to a saturated or unsaturated ring, e.g., cyclohexane, cyclopentane, or cyclohexene. Any combination of saturated, unsaturated and aromatic bicyclic rings, as valence permits, is included in the definition of carbocyclic. Exemplary "carbocycles" include cyclopentane, cyclohexane, bicyclo[2.2.1]heptane, 1,5-cyclooctadiene, 1,2,3,4-tetrahydronaphthalene, bicyclo[4.2.0]oct-3-ene, naphthalene and adamantane. Exemplary fused carbocycles include decalin, naphthalene, 1,2,3,4-tetrahydronaphthalene, bicyclo[4.2.0]octane, 4,5,6,7-tetrahydro-1H-indene and bicyclo[4.1.0]hept-3-ene. "Carbocycles" may be substituted at any one or more positions capable of bearing a hydrogen atom.

A "cycloalkyl" group is a cyclic hydrocarbon which is completely saturated. "Cycloalkyl" includes monocyclic and bicyclic rings. Typically, a monocyclic cycloalkyl group has from 3 to about 10 carbon atoms, more typically 3 to 8 carbon atoms unless otherwise defined. The second ring of a bicyclic cycloalkyl may be selected from saturated, unsaturated and aromatic rings. Cycloalkyl includes bicyclic molecules in which one, two or three or more atoms are shared between the two rings. The term "fused cycloalkyl" refers to a bicyclic cycloalkyl in which each of the rings shares two adjacent atoms with the other ring. The second ring of a fused bicyclic cycloalkyl may be selected from saturated, unsaturated and aromatic rings. A "cycloalkenyl" group is a cyclic hydrocarbon containing one or more double bonds.

The term "carbocyclylalkyl", as used herein, refers to an alkyl group substituted with a carbocycle group.

The term "carbonate" is art-recognized and refers to a group $-OCO_2-R^A$, wherein R^A represents a hydrocarbyl group.

The term "carboxy", as used herein, refers to a group represented by the formula $-CO_2H$.

The term "diazo", as used herein, refers to a group represented by the formula =N=N.

The term "disulfide" is art-recognized and refers to a group $-S-S-R^A$, wherein R^A represents a hydrocarbyl group.

The term "enol ester", as used herein, refers to a group $-C(O)O-C(R^A)=C(R^A)_2$ wherein R^A represents a hydrocarbyl group.

The term “ester”, as used herein, refers to a group -C(O)OR^A wherein R^A represents a hydrocarbyl group.

The term “ether”, as used herein, refers to a hydrocarbyl group linked through an oxygen to another hydrocarbyl group. Accordingly, an ether substituent of a hydrocarbyl group may be hydrocarbyl-O-. Ethers may be either symmetrical or unsymmetrical. Examples of ethers include, but are not limited to, heterocycle-O-heterocycle and aryl-O-heterocycle. Ethers include “alkoxyalkyl” groups, which may be represented by the general formula alkyl-O-alkyl.

The terms “halo” and “halogen” as used herein means halogen and includes chloro, fluoro, bromo, and iodo.

The terms “hetaralkyl” and “heteroaralkyl”, as used herein, refers to an alkyl group substituted with a hetaryl group.

The term "heteroalkyl", as used herein, refers to a saturated or unsaturated chain of carbon atoms and at least one heteroatom, wherein no two heteroatoms are adjacent.

The terms “heteroaryl” and “hetaryl” include substituted or unsubstituted aromatic single ring structures, preferably 5- to 7-membered rings, more preferably 5- to 6-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms “heteroaryl” and “hetaryl” also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heteroaromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, and pyrimidine, and the like.

The term “heteroatom” as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, and sulfur.

The terms “heterocyclyl”, “heterocycle”, and “heterocyclic” refer to substituted or unsubstituted non-aromatic ring structures, preferably 3- to 10-membered rings, more preferably 3- to 7-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms “heterocyclyl” and “heterocyclic” also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heterocyclic, e.g., the other cyclic rings can be cycloalkyls,

cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heterocyclyl groups include, for example, piperidine, piperazine, pyrrolidine, tetrahydropyran, tetrahydrofuran, morpholine, lactones, lactams, and the like.

The term "heterocyclylalkyl", as used herein, refers to an alkyl group substituted with a heterocycle group.

The term "hydrocarbyl", as used herein, refers to a group that is bonded through a carbon atom that does not have a =O or =S substituent, and typically has at least one carbon-hydrogen bond and a primarily carbon backbone, but may optionally include heteroatoms. Thus, groups like methyl, ethoxyethyl, 2-pyridyl, and trifluoromethyl are considered to be hydrocarbyl for the purposes of this application, but substituents such as acetyl (which has a =O substituent on the linking carbon) and ethoxy (which is linked through oxygen, not carbon) are not. Hydrocarbyl groups include, but are not limited to aryl, heteroaryl, carbocycle, heterocyclyl, alkyl, alkenyl, alkynyl, and combinations thereof.

The term "hydroxyalkyl", as used herein, refers to an alkyl group substituted with a hydroxy group.

The term "lower" when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups where there are ten or fewer non-hydrogen atoms in the substituent, preferably six or fewer. A "lower alkyl", for example, refers to an alkyl group that contains ten or fewer carbon atoms, preferably six or fewer. In certain embodiments, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy substituents defined herein are respectively lower acyl, lower acyloxy, lower alkyl, lower alkenyl, lower alkynyl, or lower alkoxy, whether they appear alone or in combination with other substituents, such as in the recitations hydroxyalkyl and aralkyl (in which case, for example, the atoms within the aryl group are not counted when counting the carbon atoms in the alkyl substituent).

The term "orthoester" as used herein is art-recognized and refers to a group – $C(OR^A)_3$, wherein each R^A independently represents hydrogen or a hydrocarbyl, such as alkyl, or any occurrence of R^A taken together with another and the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

The term "phosphoester", as used herein, refers to a group -$P(O_2)OH$.

The term "phosphodiester", as used herein, refers to a group -$P(O_2)OR^A$ wherein R^A represents a hydrocarbyl group.

The terms "polycyclyl", "polycycle", and "polycyclic" refer to two or more rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls) in which two or more atoms are common to two adjoining rings, e.g., the rings are "fused rings". Each of the rings of the polycycle can be substituted or unsubstituted. In certain embodiments, each ring of the polycycle contains from 3 to 10 atoms in the ring, preferably from 5 to 7.

The term "selenide", as used herein, is equivalent to an ether, wherein the oxygen is replaced with a selenium.

The term "selenoxide" is art-recognized and refers to the group -Se(O)-R^A, wherein R^A represents a hydrocarbyl.

The term "siloxane" is art-recognized and refers to a group with an Si-O-Si linkage, such as the group $-Si(R^A)_2$-O-Si-$(R^A)_3$, wherein each R^A independently represents hydrogen or hydrocarbyl, such as alkyl, or both R^A taken together with the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

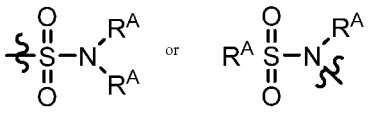
The term "silyl" refers to a silicon moiety with three hydrocarbyl moieties attached thereto.

The term "substituted" refers to moieties having substituents replacing a hydrogen on one or more carbons of the backbone. It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and non-aromatic substituents of organic compounds. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. Substituents can include any substituents described herein, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxy, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a

sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. In preferred embodiments, the substituents on substituted alkyls are selected from C_{1-6} alkyl, C_{3-6} cycloalkyl, halogen, carbonyl, cyano, or hydroxyl. In more preferred embodiments, the substituents on substituted alkyls are selected from fluoro, carbonyl, cyano, or hydroxyl. It will be understood by those skilled in the art that substituents can themselves be substituted, if appropriate. Unless specifically stated as "unsubstituted," references to chemical moieties herein are understood to include substituted variants. For example, reference to an "aryl" group or moiety implicitly includes both substituted and unsubstituted variants.

The term "sulfate" is art-recognized and refers to the group $-OSO_3H$, or a pharmaceutically acceptable salt thereof.

The term "sulfonamide" is art-recognized and refers to the group represented by the general formulae



wherein each R^A independently represents hydrogen or hydrocarbyl, such as alkyl, or both R^A taken together with the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

The term "sulfoxide" is art-recognized and refers to the group $-S(O)-R^A$, wherein R^A represents a hydrocarbyl.

The term "sulfonate" is art-recognized and refers to the group SO_3H, or a pharmaceutically acceptable salt thereof.

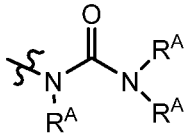
The term "sulfone" is art-recognized and refers to the group $-S(O)_2-R^A$, wherein R^A represents a hydrocarbyl.

The term "thioalkyl", as used herein, refers to an alkyl group substituted with a thiol group.

The term "thioester", as used herein, refers to a group $-C(O)SR^A$ or $-SC(O)R^A$ wherein R^A represents a hydrocarbyl.

The term "thioether", as used herein, is equivalent to an ether, wherein the oxygen is replaced with a sulfur.

The term "urea" is art-recognized and may be represented by the general formula



wherein each R^A independently represents hydrogen or a hydrocarbyl, such as alkyl, or any occurrence of R^A taken together with another and the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

"Protecting group" refers to a group of atoms that, when attached to a reactive functional group in a molecule, mask, reduce or prevent the reactivity of the functional group. Typically, a protecting group may be selectively removed as desired during the course of a synthesis. Examples of protecting groups can be found in Greene and Wuts, *Protective Groups in Organic Chemistry,* 3rd Ed., 1999, John Wiley & Sons, NY and Harrison et al., *Compendium of Synthetic Organic Methods*, Vols. 1-8, 1971-1996, John Wiley & Sons, NY. Representative nitrogen protecting groups include, but are not limited to, formyl, acetyl, trifluoroacetyl, benzyl, benzyloxycarbonyl ("CBZ"), tert-butoxycarbonyl ("Boc"), trimethylsilyl ("TMS"), 2-trimethylsilyl-ethanesulfonyl ("TES"), trityl and substituted trityl groups, allyloxycarbonyl, 9-fluorenylmethyloxycarbonyl ("FMOC"), nitro-veratryloxycarbonyl ("NVOC") and the like. Representative hydroxyl protecting groups include, but are not limited to, those where the hydroxyl group is either acylated (esterified) or alkylated such as benzyl and trityl ethers, as well as alkyl ethers, tetrahydropyranyl ethers, trialkylsilyl ethers (e.g., TMS or TIPS groups), glycol ethers, such as ethylene glycol and propylene glycol derivatives and allyl ethers.

The term "modulate" as used herein includes the inhibition or suppression of a function or activity (such as cell proliferation) as well as the enhancement of a function or activity.

The phrase "pharmaceutically acceptable" is art-recognized. In certain embodiments, the term includes compositions, excipients, adjuvants, polymers and other materials and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

"Pharmaceutically acceptable salt" or "salt" is used herein to refer to an acid addition salt or a basic addition salt which is suitable for or compatible with the treatment of patients.

The term "pharmaceutically acceptable acid addition salt" as used herein means any non-toxic organic or inorganic salt of any base compounds represented by Formula I. Illustrative inorganic acids which form suitable salts include hydrochloric, hydrobromic, sulfuric and phosphoric acids, as well as metal salts such as sodium monohydrogen orthophosphate and potassium hydrogen sulfate. Illustrative organic acids that form suitable salts include mono-, di-, and tricarboxylic acids such as glycolic, lactic, pyruvic, malonic, succinic, glutaric, fumaric, malic, tartaric, citric, ascorbic, maleic, benzoic, phenylacetic, cinnamic and salicylic acids, as well as sulfonic acids such as p-toluene sulfonic and methanesulfonic acids. Either the mono or di-acid salts can be formed, and such salts may exist in either a hydrated, solvated or substantially anhydrous form. In general, the acid addition salts of compounds of Formula I are more soluble in water and various hydrophilic organic solvents, and generally demonstrate higher melting points in comparison to their free base forms. The selection of the appropriate salt will be known to one skilled in the art. Other non-pharmaceutically acceptable salts, e.g., oxalates, may be used, for example, in the isolation of compounds of Formula I for laboratory use, or for subsequent conversion to a pharmaceutically acceptable acid addition salt.

The term "pharmaceutically acceptable basic addition salt" as used herein means any non-toxic organic or inorganic base addition salt of any acid compounds represented by Formula I or any of their intermediates. Illustrative inorganic bases which form suitable salts include lithium, sodium, potassium, calcium, magnesium, or barium hydroxide. Illustrative organic bases which form suitable salts include aliphatic, alicyclic, or aromatic organic amines such as methylamine, trimethylamine and picoline or ammonia. The selection of the appropriate salt will be known to a person skilled in the art.

Many of the compounds useful in the methods and compositions of this disclosure have at least one stereogenic center in their structure. This stereogenic center may be present in a R or a S configuration, said R and S notation is used in correspondence with the rules described in Pure Appl. Chem. (1976), 45, 11-30. The disclosure contemplates all stereoisomeric forms such as enantiomeric and diastereoisomeric forms of the compounds, salts, prodrugs or mixtures thereof (including all possible mixtures of stereoisomers). See, e.g., WO 01/062726.

Furthermore, certain compounds which contain alkenyl groups may exist as Z (zusammen) or E (entgegen) isomers. In each instance, the disclosure includes both mixture and separate individual isomers.

Some of the compounds may also exist in tautomeric forms. Such forms, although not explicitly indicated in the formulae described herein, are intended to be included within the scope of the present disclosure.

"Prodrug" or "pharmaceutically acceptable prodrug" refers to a compound that is metabolized, for example hydrolyzed or oxidized, in the host after administration to form the compound of the present disclosure (e.g., compounds of formula I). Typical examples of prodrugs include compounds that have biologically labile or cleavable (protecting) groups on a functional moiety of the active compound. Prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, hydroxylated, dehydroxylated, hydrolyzed, dehydrolyzed, alkylated, dealkylated, acylated, deacylated, phosphorylated, or dephosphorylated to produce the active compound. Examples of prodrugs using ester or phosphoramidate as biologically labile or cleavable (protecting) groups are disclosed in U.S. Patents 6,875,751, 7,585,851, and 7,964,580, the disclosures of which are incorporated herein by reference. The prodrugs of this disclosure are metabolized to produce a compound of Formula I. The present disclosure includes within its scope, prodrugs of the compounds described herein. Conventional procedures for the selection and preparation of suitable prodrugs are described, for example, in "Design of Prodrugs" Ed. H. Bundgaard, Elsevier, 1985.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filter, diluent, excipient, solvent or encapsulating material useful for formulating a drug for medicinal or therapeutic use.

The term "Log of solubility", "LogS" or "logS" as used herein is used in the art to quantify the aqueous solubility of a compound. The aqueous solubility of a compound significantly affects its absorption and distribution characteristics. A low solubility often goes along with a poor absorption. LogS value is a unit stripped logarithm (base 10) of the solubility measured in mol/liter.

EXAMPLES

The invention now being generally described, it will be more readily understood by reference to the following examples which are included merely for purposes of illustration of

certain aspects and embodiments of the present invention, and are not intended to limit the invention.

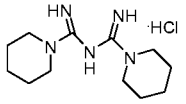
Example 1: Synthetic Procedures

All the solvents or reagents were purified according to literature procedures. High Resolution Mass Spectrometry was obtained on a Waters LCT Premier XE Time of Flight LC-MS. ^{1}H NMR, ^{13}C NMR spectra were obtained on AV-300, ARX-400, ARX-500 or Avance-500 spectrometers. The chemical shifts are reported in parts per million (ppm, d). The coupling constants are reported in Hertz (Hz) and the resonance patterns are reported with the following notations: br (broad), s (singlet), d (double), t (triplet), q (quartet) and m (multiplet). High-resolution mass spectra were measured on a time-of-flight LC-MS. Thin-layer chromatography (TLC) was carried out using precoated silica gel sheets (Merck 60 F_{254}). Visual detection was performed with ultraviolet light (short wave and long wave), *p*-anisaldehyde stain, and potassium permanganate stain.

General procedures for preparation of metformin analogues are provided in U.S. Patent Pub. No. 2015/0158832, which is incorporated by reference herein in its entirety. Exemplary procedures are provided below.

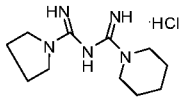
Symmetrical metformin analogues (002 and **006)**: A mixture of sodium dicyanoamide (1.335 g, 0.015 mol) and the desired secondary amine hydrochloride (0.030 mol) in xylenes (40 mL) was refluxed for 48 h. After it was cooled to 21 °C, the reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in hot MeOH and filtered. The filtrate was concentrated followed by recrystallization two or three times to afford the desired products, **002** and **006**.

Asymmetrical analogues (005): A mixture of sodium dicyanoamide (1.335 g, 0.015 mol) and pyrrolidine hydrochloride or dimethylamine hydrochloride (0.015 mol) in xylenes (40 mL) was refluxed for 12 h. After it was cooled to 21 °C, the reaction mixture was evaporated to dryness under reduced pressure. The residue was crystallized from MeOH to get the pure alkyldicyandiamide product as an intermediate. The alkyldicyandiamide (0.015 mol) was then dissolved in xylenes (40 mL), and then either piperidine hydrochloride or morpholine hydrochloride (0.015 mol) was added and the mixture was refluxed for another 48 h. The solvent was removed under vacuum and the crude residue was recrystallized twice from MeOH to provide the desired product **005**.



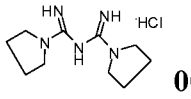
002

N-(Imino(piperidin-1-yl)methyl)piperidine-1-carboximidamide hydrochloride, 002, was obtained by following the general procedure for symmetric analogues in the presence of piperidine hydrochloride in 38% yield after crystallization from MeOH. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.10 (1H, s), 3.38 (8H, t, J = 15 Hz), 1.48 (12H, m). ^{13}C NMR (75 MHz, DMSO-d_6): δ 157.1, 45.6, 25.0, 23.7. HRMS (ESI) calcd for $[C_{12}H_{23}N_5+H]^+$ 238.2032, found 238.2026.



005

N-(Imino(pyrrolidin-1-yl)methyl)piperidine-1-carboximidamide hydrochloride, 005, was obtained by following the general procedure for asymmetric analogues in the presence of pyrrolidine hydrochloride and then piperidine hydrochloride in 35% yield after crystallization from MeOH. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.88 (1H, s), 3.40 (4H, br s), 3.29 (4H, br s), 1.83 (4H, br s), 1.54 (2H, br s), 1.48 (4H, br s). ^{13}C NMR (75 MHz, DMSO-d_6): δ 156.4, 156.1, 46.9, 45.6, 25.1, 24.8, 23.7. HRMS (ESI) calcd for $[C_{11}H_{21}N_5+H]^+$ 224.1875, found 224.1876.



006

N-(Imino(pyrrolidin-1-yl)methyl)pyrrolidine-1-carboximidamide hydrochloride, 006, was obtained by following the general procedure for symmetric analogues in the presence of pyrrolidine hydrochloride in 42% yield after crystallization from MeOH. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.97 (1H, s), 3.30 (8H, br s), 1.83 (8H, br s). ^{13}C NMR (75 MHz, DMSO-d_6): δ 155.5, 46.9, 24.8. HRMS (ESI) calcd for $[C_{10}H_{19}N_5+H]^+$ 210.1719, found 210.1717.

Example 2: Metformin analogues alone and combined with immune checkpoint inhibitors suppress TNBC progression in vivo .

In view of promising antitumor effects of analogues 002, 005 and 006 *in vitro*, analogue 005 was tested for antitumor effects using human TNBC xenografts in nude mouse models *in vivo* as compared with that of metformin. Results show that the analogue

significantly outperforms metformin in blocking TNBC progression (**FIG. 1A**). Next, a pilot study was conducted to assess antitumor activity of analogue 006 alone or combined with an anti-PD-L1 immune checkpoint inhibitor. Murine mammary carcinoma 4T1 TNBC cells were implanted in immune-competent syngeneic BALB/c mice (**FIG. 1B**). Analogue 006 alone or with anti-PD-L1 suppresses TNBCs *in vivo* as compared to control, and analogue 006 combined with anti-PD-L1 antibody therapy exhibits greater suppression of 4T1 tumor growth than that of anti-PD-L1 antibody given alone ($P<0.001$).

Example 3: $CD8^+$ T-cells and new biguanides.

Metformins are reported to suppress tumor growth *in vivo* in part by modulating $CD8^+$ T cells (7). Hence, from the same experiment described in Example 2, the effect of biguanide 006 was assessed on the number of $CD8^+$ T-cells present in mouse tumors harvested at day 10 after treatment. Cells were isolated and processed from primary 4T1 tumors by established methods, then stained with cell surface biomarkers and analyzed by mass cytometry (cyTOF). FIG. 2A shows an apparent increase in the occurance of $CD8^+$ T-cells in 4T1 tumors of mice treated with biguanide 006 as compared to that of tumors treated with vehicle. In addition, we processsed 4T1 tumor specimens in parallel to perform immunohistochemistry, and these findings confirm that increased numbers of CD8+ T-cells occur in the tumor microenvironment (not shown).

The presence of intratumoral T-cells is an independent predictor of improved survival (19,20) and has also been associated with a Th1 cytokine signature in malignant CD8+ T-cells stimulated by cancer cell antigens, with increased secretion of IFNγ, IL-2 and TNFα. Importantly, further investigations of CD8+ T-cell biomarkers using cyTOF indicate that these immune cell subsets also show evidence of biologic activation when exposed to metformin analogue 006, anti-PD-L1 antibody or the combination treatment as compared to the control-treated group (**FIGs. 2B and 2C**; $P<0.01$; $n=5$). Additional markers such as TNFα, IL-4, IL-2 and $CD69^{high}$-expressing cells are being explored in ongoing work.

Example 4: Myeloid-derived suppressor cells and new biguanides.

Tumors generate a suppressive microenvironment to evade the immune response by various mechanisms, including recruitment of regulatory T cells (T_{regs}) and myeloid-derived suppressor cells (MDSC) (12). These cell subtypes have emerged as key contributors to tumor immune suppression, tumor angiogenesis, drug resistance and promotion of tumor

metastases (13, 14). In malignancy, myeloid cell differentiation into mature macrophage, dendritic cells and granulocytes is often diverted into pathways that favor the differentiation of pathological MDSC.

The effects of biguanide 006 on the expansion of MDSC populations were assessed. Two main subpopulations of MDSCs have been characterized previously: myelomonocytic MDSC (M-MDSC) and granulocytic MDSC (G-MDSC), also known as polymorphonuclear MDSC (15, 16). In mice that bear tumors, the prevalent subpopulation of MDSCs is the G-MDSC subset which suppress antigen-specific $CD8^+$ TILs (15, 16). FIGs. 3A-3B show the results of an experiment that illustrates the G-MDSC subpopulations in tumors of mice, following treatment protocols described in Example 2. Mice were sacrificed at day 10 post-treatment, with tumors and spleens harvested. Briefly, tissues were collected following approved guidelines, with single-cell suspensions generated using a tumor dissociation kit. Tissues were minced into 2-mm pieces and mixed with recommended enzymes as per standard protocols (21). Single cell suspensions were stained using a cocktail of antibodies such as $CD11b^+Lys6G^{hi}Ly6C^{lo}$ for MDSCs, FoxP3 for Tregs, and others, including previous staining with cisplatin to discriminate live and dead cells. Analyses were then done by mass cytometry (cyTOF). G-MDSCs were stained and analyzed by cyTOF to discriminate between immune cell subpopulations, with G-MDSC defined as $CD45^+CD11b^+Ly6G^{hi}$ cells.

FIG. 3A shows a graphic representation of a viSNE clustering analysis using Cytobank defines populations of distinct immune cells from tumors and spleens. Equal event sampling using 10,000 events per individual sample was used. Cellular phenotypes were assigned to the viSNE plot based on distribution and expression characteristics using phenotypic markers: B cells ($CD19^+CD220^+$), $CD3^+CD4^+$ and $CD3^+CD8^+$ TILs, Dendritic Cells (DC, $CD11c^+MHCII^+$), Natural Killer cells (NK, $CD335^+CD161^+$), G-MDSC ($CD11b^+Ly6G^{hi}Ly6C^{low}$) M-MDSC ($CD11b^+Ly6C^{hi}Ly6G^{low}$). Expression of G-MDSC (CD11b+ Ly6G+) in the gated CD45+ cells from tumors were measured by mass cytometry. Representative plots are shown in **FIG. 3B**, N=5. A representative example of the gating strategy used in these cyTOF experiments is shown in **FIG. 3C**.

These findings are consistent with independent studies that highlight MDSCs as valuable biomarkers and as potential therapeutic targets in malignancies (16, 17). The inhibition of suppressive activities towards $CD8^+$ T cells in addition to inducing marked changes in the tumor microenvironment with respect to $CD8^+$ TILs (7), provides evidence of synergism between the novel biguanides and immune checkpoint inhibitors.

Example 5: Bone marrow isolates from breast cancer patients.

Further confirmation of the potential importance of biguanide 006 in the regulation of MDSCs was obtained in experiments using bone marrow isolates from breast cancer patients (14-21). Results shown in FIG. 4 indicate that biguanide 006 inhibits the expansion of human MDSCs derived from bone marrow specimens provided by breast cancer patients.

INCORPORATION BY REFERENCE

All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

1. Liu B, Fan Z, Edgerton SM, Deng X-S, Alimova IN, Lind SE, Thor AD (2009) Metformin induces unique biological and molecular responses in triple negative breast cancer cells. Cell Cycle 8(13):2031-2040.

2. Taubes G (2012) Unraveling the Obesity-Cancer Connection. Science 335(6064):28-32.

3. Koh M, Lee J-C, Min C, Moon A (2013) A novel metformin derivative, HL010183, inhibits proliferation and invasion of triple-negative breast cancer cells. Bioorg. Med. Chem. 21(8):2305-2313.

4. Evans JMM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD (2005) Metformin and reduced risk of cancer in diabetic patients. Brit. Med. J. 330(7503):1304-1305.

5. Dowling RJO, Niraula S, Chang MC, Done SJ, Ennis M, McCready DR, Leong WL, Escallon JM, Reedijk M, Goodwin PJ, Stambolic V (2015) Changes in insulin receptor signaling underlie neoadjuvant metformin administration in breast cancer: a prospective window of opportunity neoadjuvant study. Breast Cancer Res 17(1):32.

6. Jiralerspong S, Palla SL, Giordano SH, Meric-Bernstam F, Liedtke C, Barnett CM, Hsu L, Hung M-C, Hortobagyi GN, Gonzalez-Angulo AM (2009) Metformin and Pathologic Complete Responses to Neoadjuvant Chemotherapy in Diabetic Patients With Breast Cancer. J. Clin. Oncol. 27(20):3297-3302.

7. Eikawa S, Nishida M, Mizukami S, Yamazaki C, Nakayama E, Udono H (2015) Immune-mediated antitumor effect by type 2 diabetes drug, metformin. Proc. Natl. Acad. Sci. U.S.A. 112(6):1809-1814.

8. Topalian Suzanne L, Drake Charles G, Pardoll Drew M (2015) Immune Checkpoint Blockade: A Common Denominator Approach to Cancer Therapy. Cancer Cell 27(4):450-461.

9. Cimino-Mathews A, Foote J, Emens LA (2015) Immune Targeting in Breast Cancer. Oncology 29(5):375-385.

10. Bailey-Downs LC, Thorpe JE, Disch BC, Bastian A, Hauser PJ, Farasyn T, Berry WL, Hurst RE, Ihnat MA (2014) Development and Characterization of a Preclinical Model of Breast Cancer Lung Micrometastatic to Macrometastatic Progression. PLoS One 9(5):e98624.

11. Escuin-Ordinas H, Elliott MW, Atefi M, Lee M, Ng C, Wei L, Comin-Anduix B, Montecino-Rodriguez E, Avramis E, Radu C, Sharp LL, Ribas A (2013) PET imaging to non-invasively study immune activation leading to antitumor responses with a 4-1BB agonistic antibody. J. Immunother. Cancer 1:14-24.

12. Gajewski TF, Woo S-R, Zha Y, Spaapen R, Zheng Y, Corrales L, Spranger S (2013) Cancer immunotherapy strategies based on overcoming barriers within the tumor microenvironment. Curr. Opin. Immunol. 25(2):268-276.

13. Lindau D, Gielen P, Kroesen M, Wesseling P, Adema GJ (2013) The immunosuppressive tumour network: myeloid-derived suppressor cells, regulatory T cells and natural killer T cells. Immunology 138(2):105-115.

14. Gabrilovich DI (2017) Myeloid-Derived Suppressor Cells. Cancer Immunol. Res. 5(1):3-8.

15. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V (2012) Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol 12:253-268.

16. Marvel D, Gabrilovich DI (2015) Myeloid-derived suppressor cells in the tumor microenvironment: expect the unexpected. J. Clin. Invest. 125(9):3356-3364.

17. Wesolowski R, Markowitz J, Carson WE (2013) Myeloid derived suppressor cells – a new therapeutic target in the treatment of cancer. J. Immunother. Cancer 1(1):10.

18. Ruffell B, Au A, Rugo HS, Esserman LJ, Hwang ES, Coussens LM (2012). Leukocyte composition of human breast cancer. Proc Natl Acad Sci U S A. 109(8):2796-801.

19. Stanton SE, Adams S, Disis ML. Variation in the Incidence and Magnitude of Tumor-Infiltrating Lymphocytes in Breast Cancer Subtypes: A Systematic Review. JAMA Oncol. 2016;2:1354-1360.

20. Siska PJ, Rathmell JC. T cell metabolic fitness in antitumor immunity. Trends Immunol. 2015;36:257-64. PMCID: PMC4393792.

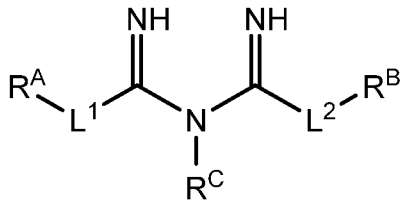
21. Hu-Lieskovan S, Mok S, Homet Moreno B, Tsoi J, Robert L, Goedert L, Pinheiro EM, Koya RC, Graeber TG, Comin-Anduix B, Ribas A. Improved antitumor activity of immunotherapy with BRAF and MEK inhibitors in BRAF V600E melanoma. Sci. Transl. Med. 2015; 7:241-79. PMCID:PMC4765379.

EQUIVALENTS

While specific embodiments of the subject invention have been discussed, the above specification is illustrative and not restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification and the claims below. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

We claim:

1. A pharmaceutical composition comprising a compound of formula A, or a pharmaceutically acceptable salt thereof, and an immune therapy:



(A)

wherein:

L^1 and L^2 are independently a bond or -NH-C(NH)-;

R^A is $-NR^1R^2$, alkyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl; R^1 and R^2 taken together with the N that separate them complete a heterocycle;

R^B is $-NR^3R^4$, alkyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl; or R^3 and R^4 taken together with the N that separate them complete a heterocycle;

R^1 is hydrogen, $-OR^5$, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl;

R^2 is hydrogen, $-OR^5$, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl; or ; R^1 and R^2 taken together with the N that separates them complete a heterocycle;

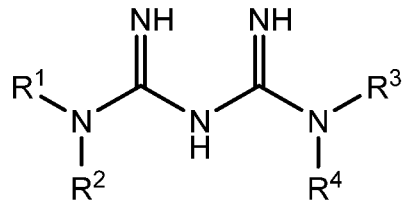
R^3 is hydrogen, $-OR^5$, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl;

R^4 is hydrogen, $-OR^5$, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl; or ; R^3 and R^4 taken together with the N that separates them complete a heterocycle;

R^C is hydrogen or C_1-C_5 alkyl; and

R^5 is hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl.

2. The pharmaceutical composition of claim 1, wherein the compound of formula A is a compound of formula I:



(I).

3. The pharmaceutical composition of any one of the preceding claims, wherein:

R^1 is H, alkyl or alkoxy;

R^2 is H, alkyl or alkoxy; or R^1 and R^2, taken together, complete a 5-10 member heterocycle;

R^3 is H, alkyl or alkoxy; and
R^4 is H, alkyl or alkoxy; or R^3 and R^4, taken together, complete a 5-10 member heterocycle.

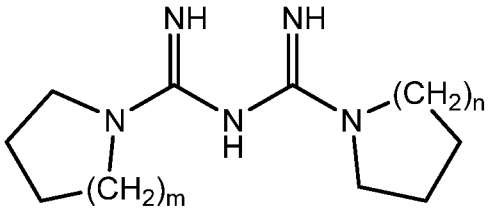
4. The pharmaceutical composition of any one of the preceding claims, wherein if R^1 and R^2 are methyl, then R^3 and R^4 are not both hydrogen.

5. The pharmaceutical composition of any one of claims 1-4, wherein R^1 is methoxy and R^2 is methyl.

6. The pharmaceutical composition of any one of claims 1-4, wherein R^1 is propyl and R^2 is methyl.

7. The pharmaceutical composition of any one of the preceding claims, wherein R^3 and R^4 are H.

8. The pharmaceutical composition of any one of claims 1-3, wherein the compound is of formula Ia, or a pharmaceutically acceptable salt thereof:



(Ia)

wherein:
m is 0, 1, 2, or 3; and
n is 0, 1, 2, or 3.

9. The pharmaceutical composition of claim 8, wherein m and n are both 1.

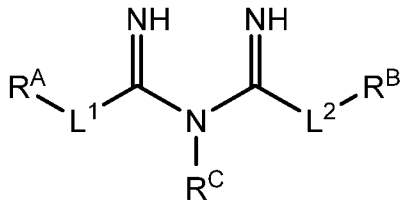
10. The pharmaceutical composition of any one of the preceding claims, wherein the immune therapy is an immune checkpoint inhibitor, an indoleamine 2,3-dioxygenase inhibitor, or an adoptive cell transfer therapy.

11. The pharmaceutical composition of any one of claims 1-10, wherein the immune therapy is an immune checkpoint inhibitor selected from anti-PD-1, anti-PD-L1, or anti-CTLA-4.

12. The pharmaceutical composition of claim 11, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, atezolizumab, durvalumab, avelumab, or ipilumumab.

13. A method for treating a cancer, comprising administering a composition of any one of the preceding claims to a patient in need thereof.

14. A method for treating a cancer, comprising administering conjointly a compound of formula A, or a pharmaceutically acceptable salt thereof, and an immune therapy:



(A)

wherein:

L^1 and L^2 are independently a bond or -NH-C(NH)-;

R^A is $-NR^1R^2$, alkyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl; R^1 and R^2 taken together with the N that separate them complete a heterocycle;

R^B is $-NR^3R^4$, alkyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl; or R^3 and R^4 taken together with the N that separate them complete a heterocycle;

R^1 is hydrogen, $-OR^5$, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl;

R^2 is hydrogen, $-OR^5$, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl; or ; R^1 and R^2 taken together with the N that separates them complete a heterocycle;

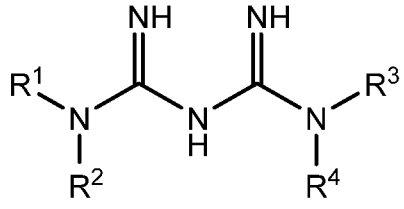
R^3 is hydrogen, $-OR^5$, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl;

R^4 is hydrogen, $-OR^5$, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl; or ; R^3 and R^4 taken together with the N that separates them complete a heterocycle;

R^C is hydrogen or C_1-C_5 alkyl; and

R^5 is hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl.

15. The method of claim 14, wherein the compound of formula A is a compound of formula I:



(I).

16. The method of any one claims 14-15, wherein:

R^1 is H, alkyl or alkoxy;

R^2 is H, alkyl or alkoxy; or R^1 and R^2, taken together, complete a 5-10 member heterocycle;

R^3 is H, alkyl or alkoxy; and

R^4 is H, alkyl or alkoxy; or R^3 and R^4, taken together, complete a 5-10 member heterocycle.

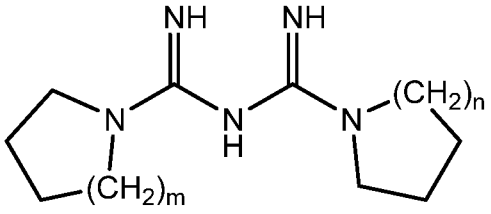
17. The method of any one of claims 14-16, wherein if R^1 and R^2 are methyl, then R^3 and R^4 are not both hydrogen.

18. The method of any one of claims 14-17, wherein R^1 is methoxy and R^2 is methyl.

19. The method of any one of claims 14-17, wherein R^1 is propyl and R^2 is methyl.

20. The method of any one of claims 14-17, wherein R^3 and R^4 are H.

21. The method of any one of claims 14-17, wherein the compound is of formula Ia, or a pharmaceutically acceptable salt thereof:



(Ia)

wherein:

m is 0, 1, 2, or 3; and

n is 0, 1, 2, or 3.

22. The method of claim 21, wherein m and n are both 1.

23. The method of any one of claims 14-22, wherein the immune therapy is an immune checkpoint inhibitor, an indoleamine 2,3-dioxygenase inhibitor, or an adoptive cell transfer therapy.

24. The method of any one of claims 14-23, wherein the immune therapy is an immune checkpoint inhibitor selected from anti-PD-1, anti-PD-L1, or anti-CTLA-4.

25. The method composition of claim 24, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, atezolizumab, durvalumab, avelumab, or ipilumumab.

26. The method of any one of claims 13-25, wherein the cancer is leukemia, lymphoma, Hodgkins lymphoma, colon cancer, breast cancer, prostate cancer, lung cancer, skin cancer, liver cancer, pancreatic cancer, ovarian cancer, bladder cancer, kidney cancer, bile duct cancer, esophageal cancer, cervical cancer, endometrial cancer, melanoma, head and neck cancer, brain cancer, glioma, neuroblastoma, osteosarcoma, chondrosarcoma, gastric carcinoma, glioma, mesothelioma, Kaposi sarcoma, liposarcoma, synovial sarcoma, or Wilm's tumor.

27. The method of claim 26, wherein the cancer is melanoma, non-small cell lung cancer, renal cell carcinoma, bladder cancer, triple-negative breast cancer, or a gynecologic cancer such as endometrial, cervical, or ovarian cancer.

28. The method of claim 27, wherein the cancer is breast cancer, such as triple-negative breast cancer.

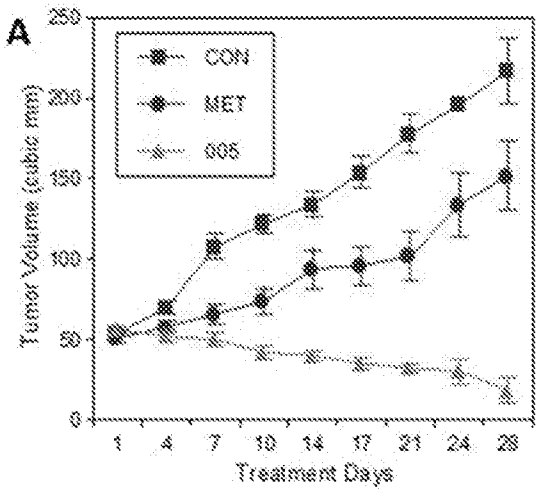

A
CON
MET
605
Tumor Volume (cubic mm)
Treatment Days
0
50
100
150
200
250
1
4
7
10
14
17
21
24
28

FIG. 1A

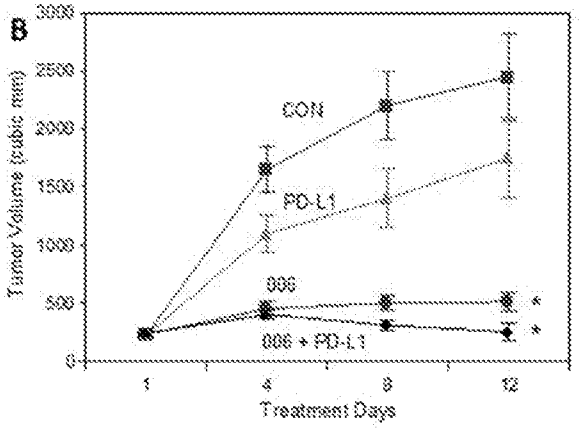

B
CON
PD-L1
606
606 + PD-L1
Tumor Volume (cubic mm)
Treatment Days
0
500
1000
1500
2000
2500
3000
1
4
8
12

FIG. 1B

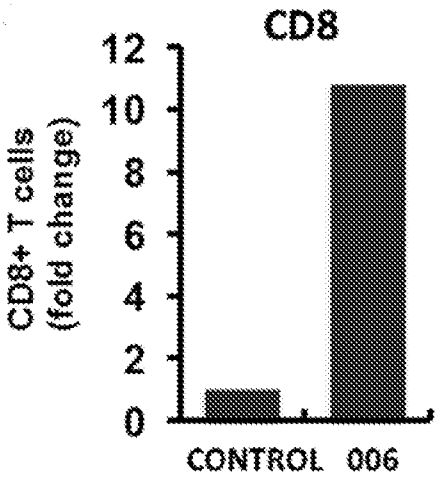


FIG. 2A

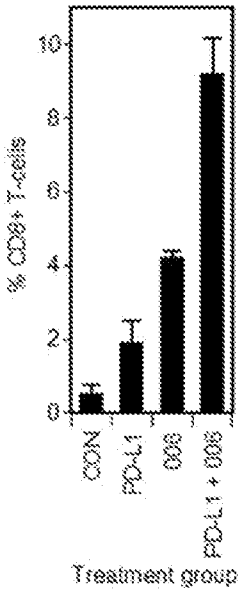

% CD8+ T-cells
0
2
4
6
8
10
CON
PD-L1
006
PD-L1 + 006
Treatment group

FIG. 2B

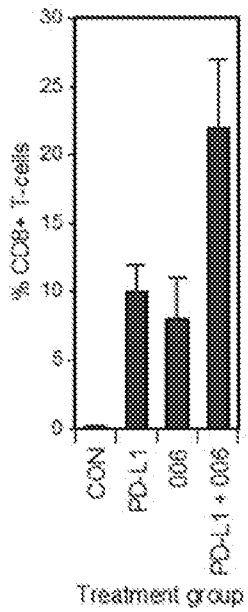

% CD8+ T-cells
0
5
10
15
20
25
30
CON
PD-L1
006
PD-L1 + 006
Treatment group

FIG. 2C

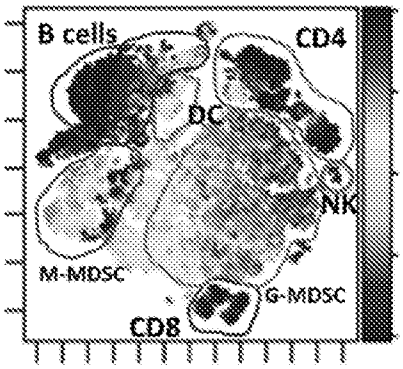

B cells
CD4
DC
NK
M-MDSC
G-MDSC
CD8

FIG. 3A

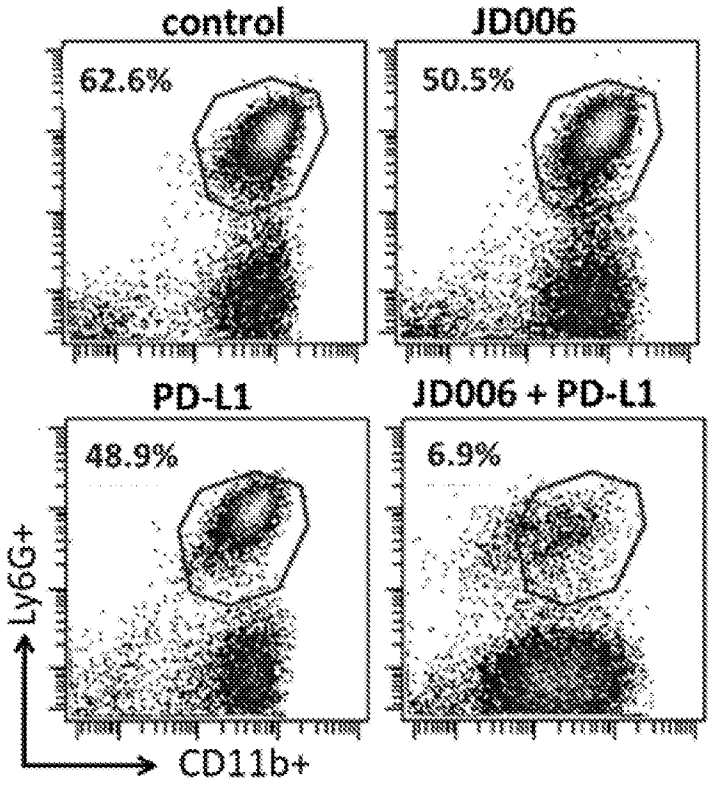

control
JD006
62.6%
50.5%
PD-L1
JD006 + PD-L1
48.9%
6.9%
Ly6G+
CD11b+

FIG. 3B

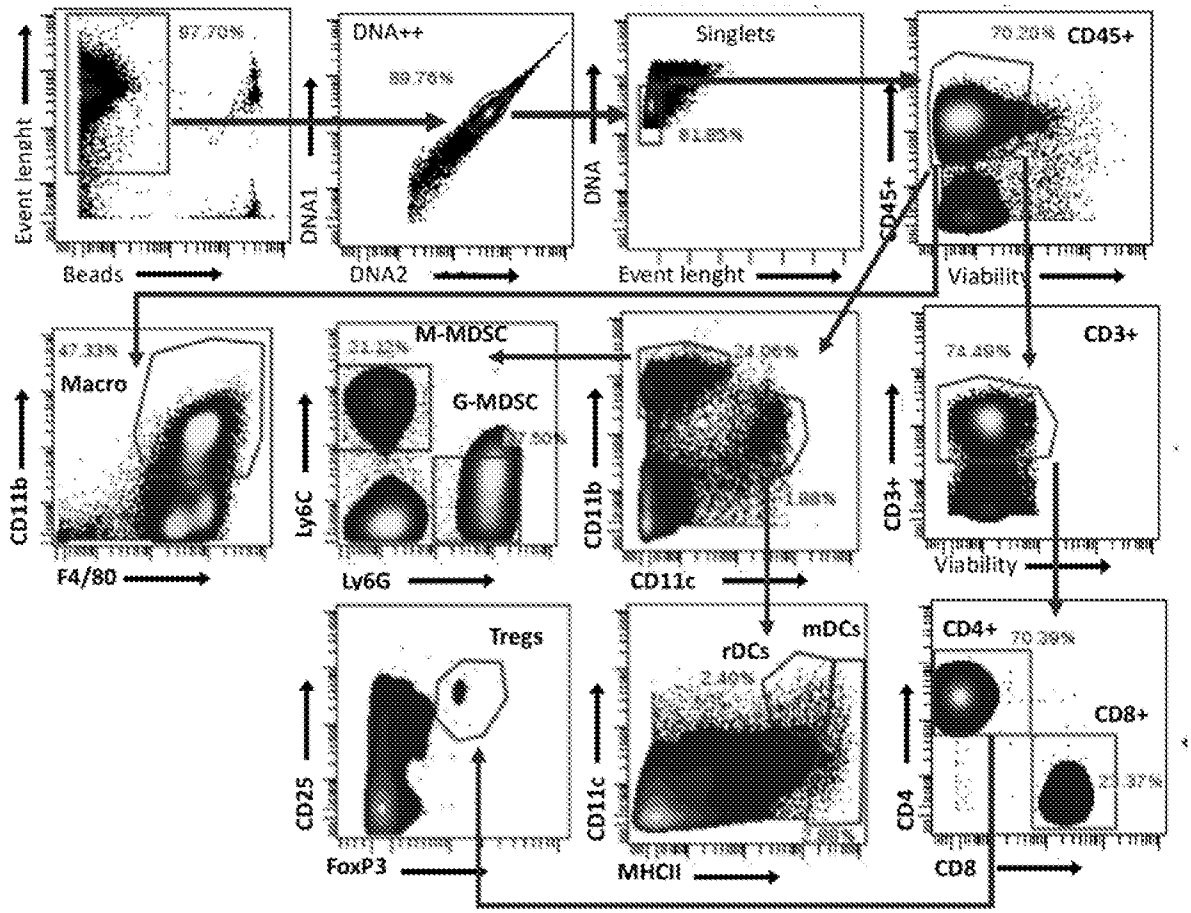

Event lenght
Beads
DNA1
DNA++
DNA2
DNA
Singlets
Event lenght
CD45+
CD45+
Viability
CD11b
Macro
F4/80
Ly6C
M-MDSC
G-MDSC
Ly6G
CD11b
CD11c
CD3+
CD3+
Viability
CD25
Tregs
FoxP3
CD11c
rDCs
mDCs
MHCII
CD4+
CD8+
CD4
CD8

FIG. 3C

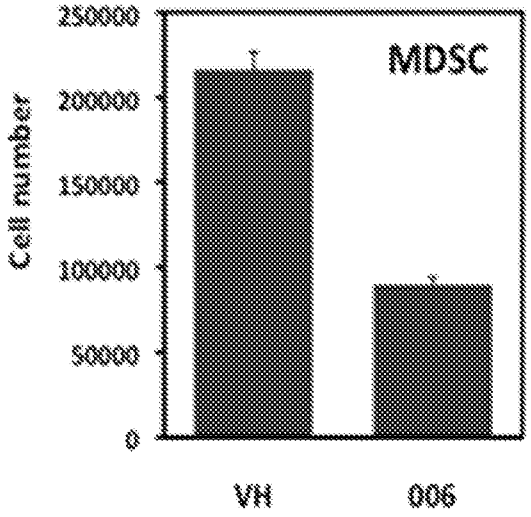


FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/063058

A. CLASSIFICATION OF SUBJECT MATTER

IPC (2019.01) A61K 31/454, A61K 31/454500, A61K 31/402500, A61P 35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC (2019.01) A61K 31/454, A61K 31/454500, A61K 31/402500

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases consulted: PATENTSCOPE, USPTO, Google Patents, CAPLUS, BIOSIS, MEDLINE, REGISTRY, Google Scholar
Search terms used: metformin, *formin, biguanidine, "anti-PD1", "anti-PD-L1", "anti-CTLA-4", "immune therapy", "immune checkpoint inhibitor", "indoleamine*dioxygenase, "adoptive cell transfer", cancer, melanoma, triple*negative, TNBC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Vazquez-Martin A. et al. The anti-diabetic drug metformin suppresses self-renewal and proliferation of trastuzumab-resistant tumor-initiating breast cancer stem cells. Breast Cancer Res. Treat. 2011 Apr; 126(2):355-64. doi: 10.1007/s10549-010-0924-x. Epub 2010 May 11. Retrieved from the Internet. URL: https://hal.archives-ouvertes.fr/hal-00583557/document. 11 May 2010 (2010/05/11) Whole document, particularly Figs. 2C, 3C and 3D.	1-3,7,13-16,20,26
Y	Whole document	1-11,13-24,26-28
X	Scharping N.E., et al. Efficacy of PD-1 blockade is potentiated by metformin-induced reduction of tumor hypoxia. Cancer immunology research. 2017 Jan 1;5(1):9-16. Author manuscript. Published online 2016 Dec 9. Retrieved from the Internet. URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5340074/pdf/nihms835819.pdf. 09 Dec 2016 (2016/12/09) Whole document, particularly Page 7	1-3,7,10-16,20, 23-27
Y	Whole document	1-11,13-24,26-28

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 Mar 2019	26 Mar 2019

Name and mailing address of the ISA:	Authorized officer
Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Facsimile No. 972-2-5651616	GARBER Nathan Telephone No. 972-2-5651675

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/063058

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Eikawa S., et al. Immune-mediated antitumor effect by type 2 diabetes drug, metformin. Proceedings of the National Academy of Sciences. 2015 Feb 10; 112(6):1809-14. Retrieved from the Internet. URL: https://www.pnas.org/content/pnas/112/6/1809.full.pdf & Supporting information. Retrieved from the Internet. URL: https://www.pnas.org/content/pnas/suppl/2015/01/22/1417636112.DCSupplemental/pnas.201417636SI.pdf?targetid=nameddest%3DSF1 10 Feb 2015 (2015/02/10) Whole document, particularly Abstract and Fig. 5.	1-3,7,13-16,20,26, 27
Y	Whole document	1-11,13-24,26-28
A	Kim S.H., et al. Phenformin inhibits myeloid-derived suppressor cells and enhances the anti-tumor activity of PD-1 blockade in melanoma. Journal of Investigative Dermatology. 2017 Aug 1; 137(8):1740-8. 01 Aug 2017 (2017/08/01) Whole document	1-28
Y	WO 2013188452 A1 (The Regents of the University of California [US]) 19 Dec 2013 (2013/12/19) Whole document, particularly Table 1 an passages [0262]-[0264], [0237], [0084]	1-11,13-24,26-28
A	US 2015/0126518 A1 (Hanall Biopharma Co. [KR]) 07 May 2015 (2015/05/07) Whole document	1-28
P,X	Afzal M.Z., et al. Efficacy of metformin in combination with immune checkpoint inhibitors (anti-PD-1/anti-CTLA-4) in metastatic malignant melanoma. Journal for immunotherapy of cancer. 2018 Dec;6(1):64. Retrieved from the Internet. URL: https://jitc.biomedcentral.com/articles/10.1186/s40425-018-0375-1 27 Nov 2018 (2018/11/27) Whole document	1-28

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US2018/063058

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
WO 2013188452 A1	19 Dec 2013	WO 2013188452 A1	19 Dec 2013
		CA 2913736 A1	19 Dec 2013
		US 2015158832 A1	11 Jun 2015
		US 9862693 B2	09 Jan 2018
US 2015/0126518 A1	07 May 2015	NONE	