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(71) Applicant *(for all designated States except US)*: **THRESHOLD PHARMACEUTICALS, INC.** [US/US]; 1300 Seaport Boulevard, 5th Floor, Redwood City, California 94063 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **TIDMARSH, George** [US/US]; 45 Tintern Lane, Portola Valley, California 94028 (US).

(74) Agents: **APPLE, Randolph, Ted** et al.; TOWNSEND AND TOWNSEND AND CREW LLP, Two Embaracadero Center, 8th Floor, San Francisco, California 94111-3834 (US).

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(54) Title: PREVENTION OF CANCER

(57) Abstract: Methods are provided for prevention of prostate cancer and epithelial cancers by administering lonidamine and related agents.

# PREVENTION OF CANCER

## CONTINUITY

**[0001]** This application claims benefit of U.S. Provisional Application No. 60/681,067, filed May 13, 2005, and U.S. Provisional Application No. 60/587,017, filed July 8, 2004; the disclosures of which are incorporated by reference herein in their entirety.

## FIELD OF THE INVENTION

**[0002]** The invention relates to methods for preventing prostate cancer and has application in the field of medicine and allied fields including but not limited to chemistry, medicinal chemistry, and biology.

## BACKGROUND OF THE INVENTION

**[0003]** Prostate cancer is one of the most prevalent malignancies in adult males and an increasingly prevalent health problem in the United States. It is typically a slow-growing malignancy that affects mostly older men. More than 75 percent of prostate cancers are detected in men over 65 years old. It also occurs in younger men, although it is rare in men under 40 years old. There remains a serious unmet need for new methods of preventing prostate cancer as well as other cancers involving epithelial cells (e.g., breast cancer).

**[0004]** Lonidamine (also known as 1-(2,4-dichlorobenzyl)-1-H-indazole-3-carboxylic acid) is an anti-cancer drug approved for single agent use in certain countries in Europe for the treatment of cancer, including lung, breast, prostate, and brain cancer. The mechanism of action of lonidamine may involve interference with the energy metabolism of neoplastic cells by disruption of the mitochondrial membrane and by inhibition of hexokinase. Lonidamine also has anti-spermatogenic activity and has been shown to inhibit germ cell respiration. See Gatto et al., 2002; Hagen et al., 2003; Heywood et al., 1981.

**[0005]** Lonidamine has been studied for use in the treatment of advanced breast cancer. Mansi *et al.*, 1991, reports a phase II study in which lonidamine was administered in a daily divided oral dose of 600 mg. The investigators concluded that lonidamine appeared to be active against advanced breast cancer. Combination

studies of lonidamine with other agents in the treatment of advanced breast cancer have also been reported. For examples, see Iaffaioli *et al.*, 1995 (epirubicin, lonidamine, and alpha 2b interferon); Gardin *et al.*, 1996 (lonidamine, epirubicin, and cyclophosphamide); Dogliotti *et al.*, 1996 (lonidamine and epirubicin); Gebbia *et al.*, 1997 (cisplatin, epirubicin, and lonidamine); Amadori *et al.*, 1998 (lonidamine and doxorubicin); Dogliotti *et al.*, 1998 (cisplatin, epirubicin, and lonidamine); Nistico *et al.*, 1999 (epirubicin and lonidamine); Pacini *et al.*, 2000 (FEC [5-fluorouracil, epidoxorubicin and cyclophosphamide] versus EM [epidoxorubicin and mitomycin-C] with or without lonidamine); Berruti *et al.*, 2002 (epirubicin with either cisplatin or lonidamine); and Berruti *et al.*, 1997.

**[0006]** Lonidamine has also been studied in lung cancer (Joss *et al.*, 1984) in combination with radiation or anti-cancer agents. For examples, see Privitera *et al.*, 1987 (lonidamine and radiotherapy); Gallo-Curcio *et al.*, 1988 (chemotherapy or radiation therapy plus and minus LND); Giaccone *et al.*, 1989 (lonidamine versus polychemotherapy); Ianniello *et al.*, 1996 (cisplatin, epirubicin, and vindesine with or without lonidamine); Gridelli *et al.*, 1997 (VM-26 plus lonidamine); Comella *et al.*, 1999 (cisplatin, gemcitabine, and vinorelbine with or without lonidamine); DeMarinis *et al.*, 1999 (vindesine and LND); and Portalone *et al.*, 1999 (cisplatin, epidoxorubicin, vindesine, and lonidamine).

**[0007]** Lonidamine has been studied as a treatment for other cancers (see Robustelli *et al.*, 1991; and Pacilio *et al.*, 1984), including B-cell neoplasms (Robins *et al.*, 1990); advanced colorectal cancer (Passalacqua *et al.*, 1989, and Zaniboni *et al.*, 1995); advanced gastric carcinoma (Barone *et al.*, 1998); malignant glioma (Carapella *et al.*, 1989; Carapella *et al.*, 1990); metastatic cancers (Weinerman, 1990; DeAngelis *et al.*, 1989; U.S. Patent No. 5,260,327; and Weinerman *et al.*, 1986); advanced ovarian cancer (Bottalico *et al.*, 1996; DeLena *et al.*, 1997; and DeLena *et al.*, 2001); and recurrent papillary carcinomas of the urinary bladder (Giannotti *et al.*, 1984).

**[0008]** Despite the numerous studies conducted, lonidamine is still not approved for use in the treatment of cancer in the United States, Asia, and most countries in Europe. Moreover, the use of lonidamine to prevent prostate cancer or other cancers in humans has not been described. There remains a serious unmet need for new methods of preventing cancer. The present invention provides such methods,

including methods for prevention of prostate cancer using lonidamine, lonidamine analogs, and other compounds.

## SUMMARY OF THE INVENTION

**[0009]** The invention provides methods for prevention of prostate cancer and other epithelial cell cancers, such as breast cancer. In one aspect, the invention provides a method of treating a human subject to reduce the likelihood of developing prostate cancer by (a) identifying the subject as being in a prostate cancer susceptibility population and (b) administering a prophylactically effective amount of lonidamine (LND) or a lonidamine analog (LNDA) to the subject. In one embodiment the subject is diagnosed with prostatic intraepithelial neoplasia (PIN). In certain embodiments the subject has an elevated serum prostate specific antigen (PSA) level; has a rising PSA level; has a family history of prostate cancer; and/or has an elevated level of a prostate-cancer susceptibility marker. In one embodiment, the subject has undergone a prostate biopsy in which no evidence of prostate cancer was detected.

**[0010]** In a related embodiment the invention provides a method for preventing prostate cancer in a human subject comprising administering a therapeutically effective amount of an energolytic agent (EA) to said human subject in need of such treatment, wherein the energolytic agent is an agent that interferes with energy metabolism in prostate epithelial cells. In embodiments, the energolytic agent is selected from the group consisting of 2-deoxyglucose, 3-bromopyruvate, gossypol, oxamate, iodoacetate, apoptolidin, and a londamine analog.

**[0011]** In a related embodiment the invention provides a method for preventing an epithelial cancer other than prostate cancer in a human subject comprising administering lonidamine or a lonidamine analog to a subject in need of prophylaxis.

## DESCRIPTION OF THE FIGURES

**[0012]** Figure 1 shows the mean change in prostate volume in men administered lonidamine in a clinical trial. Bars = 95% confidence intervals.

**[0013]** Figure 2 shows the mean change in serum PSA levels in men administered lonidamine in a clinical trial. Bars = 95% confidence intervals.

## DETAILED DESCRIPTION OF THE INVENTION

### 1. DEFINITIONS

**[0014]** The following definitions are provided to aid in understanding the invention. Unless otherwise defined, all terms of art, notations and other scientific or medical terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the chemical and medical arts. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not be assumed to represent a substantial difference over what is generally understood in the art.

**[0015]** "Alkyl" refers to a linear saturated monovalent hydrocarbon radical or a branched saturated monovalent hydrocarbon radical having the number of carbon atoms indicated in the prefix. For example, ($C_1$-$C_8$) alkyl or $C_1$-$C_8$ alkyl includes methyl, ethyl, n-propyl, 2-propyl, n-butyl, 2-butyl, tert-butyl, pentyl, and the like. For each of the definitions herein (*e.g.*, alkyl, alkenyl, alkoxy, araalkyloxy), when a prefix is not included to indicate the number of main chain carbon atoms in an alkyl portion, the radical or portion thereof will have six or fewer main chain carbon atoms. ($C_1$-$C_6$) alkyl can be further optionally be substituted with substituents, including for example, hydroxy, amino, mono or di($C_1$-$C_6$) alkyl amino, halo, $C_2$-$C_6$ alkenyl ether, cyano, nitro, ethenyl, ethynyl, $C_1$-$C_6$ alkoxy, $C_1$-$C_6$ alkylthio, -COOH, -$CONH_2$, mono- or di($C_1$-$C_6$) alkylcarbox-amido, -$SO_2NH_2$, -$OSO_2$-($C_1$-$C_6$) alkyl, mono or di($C_1$-$C_6$) alkylsulfonamido, aryl and heteroaryl.

**[0016]** "Alkenyl" refers to a linear monovalent hydrocarbon radical or a branched monovalent hydrocarbon radical having the number of carbon atoms indicated in the prefix and containing at least one double bond, but no more than three double bonds. For example, ($C_2$-$C_6$) alkenyl includes, ethenyl, propenyl, 1,3-butadienyl and the like. Alkenyl can be further optionally be substituted with substituents, including for example, hydroxy, amino, mono or di($C_1$-$C_6$) alkyl amino, halo, $C_2$-$C_6$ alkenyl ether, cyano, nitro, ethenyl, ethynyl, $C_1$-$C_6$ alkoxy, $C_1$-$C_6$ alkylthio, -COOH, -$CONH_2$, mono- or di($C_1$-$C_6$) alkyl-carboxamido, -$SO_2NH_2$, -$OSO_2$-($C_1$-$C_6$) alkyl, mono or di($C_1$-$C_6$) alkylsulfonamido, aryl and heteroaryl.

**[0017]** "Alkylene" means a linear saturated divalent hydrocarbon radical having from one to twelve carbon atoms or a branched saturated divalent hydrocarbon radical having from one to twelve carbon atoms optionally substituted with substituents including for example, hydroxy, amino, mono or di($C_1$-$C_6$)alkyl amino, halo, $C_2$-$C_6$ alkenyl ether, cyano, nitro, ethenyl, ethynyl, $C_1$-$C_6$ alkoxy, $C_1$-$C_6$ alkylthio, -COOH, -$CONH_2$, mono- or di-($C_1$-$C_6$)alkyl-carboxamido, -$SO_2NH_2$, -$OSO_2$-($C_1$-$C_6$) alkyl, mono or di($C_1$-$C_6$) alkylsulfonamido, aryl and heteroaryl. For example alkylene includes methylene, ethylene, propylene, 2-methyl-propylene, pentylene, hexylene, and the like.

**[0018]** "Heteroalkylene" has essentially the meaning given above for alkylene except that one or more heteroatoms (i.e. oxygen, sulfur, nitrogen and/or phosphorous) may be present in the alkylene biradical. For example, heteroalkylene includes, -$CH_2OCH_2O$-,-$CH_2CH_2OCH_2CH_2$-, -$CH_2CH_2N(CH_3)CH_2CH_2$-, -$CH_2CH_2SCH_2CH_2$-, and the like.

**[0019]** "Aryl" refers to a monovalent monocyclic or bicyclic aromatic hydrocarbon radical of 6 to 10 ring atoms which is substituted independently with one to eight substituents, preferably one, two, three, four ot five substituents selected from alkyl, cycloalkyl, cycloalkylalkyl, halo, nitro, cyano, hydroxy, alkoxy, amino, acylamino, mono-alkylamino, di-alkylamino, haloalkyl, haloalkoxy, heteroalkyl, COR (where R is hydrogen, alkyl, cycloalkyl, cycloalkyl-alkyl, phenyl or phenylalkyl), -$(CR'R")_n$-COOR (where n is an integer from 0 to 5, R' and R" are independently hydrogen or alkyl, and R is hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, phenyl or phenylalkyl) or -$(CR'R")_n$-$CONR^xR^y$ (where n is an integer from 0 to 5, R' and R" are independently hydrogen or alkyl, and $R^x$ and $R^y$ are independently selected from hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, phenyl or phenylalkyl). In one embodiment, $R^x$ and $R^y$ together is cycloalkyl or heterocyclyl. More specifically the term aryl includes, but is not limited to, phenyl, biphenyl, 1-naphthyl, and 2-naphthyl, and the substituted forms thereof.

**[0020]** "Cycloalkyl" refers to a monovalent cyclic hydrocarbon radical of three to seven ring carbons. The cycloalkyl group can have one or more double bonds and can also be optionally substituted independently with one, two, three or four substituents selected from alkyl, optionally substituted phenyl, or -$C(O)R^z$ (where $R^z$ is hydrogen, alkyl, haloalkyl, amino, mono-alkylamino, di-alkylamino, hydroxy,

alkoxy, or optionally substituted phenyl). More specifically, the term cycloalkyl includes, for example, cyclopropyl, cyclohexyl, cyclohexenyl, phenylcyclohexyl, 4-carboxycyclohexyl, 2-carboxamidocyclohexenyl, 2-dimethylaminocarbonyl-cyclohexyl, and the like.

**[0021]** "Heteroalkyl" means an alkyl radical as defined herein with one, two or three substituents independently selected from cyano, $-OR^w$, $-NR^xR^y$, and $-S(O)_pR^z$ (where p is an integer from 0 to 2 ), with the understanding that the point of attachment of the heteroalkyl radical is through a carbon atom of the heteroalkyl radical. $R^w$ is hydrogen, alkyl, cycloalkyl, cycloalkyl-alkyl, aryl, aralkyl, alkoxycarbonyl, aryloxycarbonyl, carboxamido, or mono- or di-alkylcarbamoyl. $R^x$ is hydrogen, alkyl, cycloalkyl, cycloalkyl-alkyl, aryl or araalkyl. $R^y$ is hydrogen, alkyl, cycloalkyl, cycloalkyl-alkyl, aryl, araalkyl, alkoxycarbonyl, aryloxycarbonyl, carboxamido, mono- or di-alkylcarbamoyl or alkylsulfonyl. $R^z$ is hydrogen (provided that n is 0), alkyl, cycloalkyl, cycloalkyl-alkyl, aryl, araalkyl, amino, mono-alkylamino, di-alkylamino, or hydroxyalkyl. Representative examples include, for example, 2-hydroxyethyl, 2,3-dihydroxypropyl, 2-methoxyethyl, benzyloxymethyl, 2-cyanoethyl, and 2-methylsulfonyl-ethyl. For each of the above, $R^w$, $R^x$, $R^y$, and $R^z$ can be further substituted by amino, halo, fluoro, alkylamino, di-alkylamino, OH or alkoxy. Additionally, the prefix indicating the number of carbon atoms (*e.g.*, $C_1$-$C_{10}$) refers to the total number of carbon atoms in the portion of the heteroalkyl group exclusive of the cyano, $-OR^w$, $-NR^xR^y$, or $-S(O)_pR^z$ portions.

**[0022]** In one embodiment, $R^x$ and $R^y$ together is cycloalkyl or heterocyclyl.

**[0023]** "Heteroaryl" means a monovalent monocyclic, bicyclic or tricyclic radical of 5 to 12 ring atoms having at least one aromatic ring containing one, two, or three ring heteroatoms selected from N, O, or S, the remaining ring atoms being C, with the understanding that the attachment point of the heteroaryl radical will be on an aromatic ring. The heteroaryl ring is optionally substituted independently with one to eight substituents, preferably one, two, three or four substituents, selected from alkyl, cycloalkyl, cycloalkyl-alkyl, halo, nitro, cyano, hydroxy, alkoxy, amino, acylamino, mono-alkylamino, di-alkylamino, haloalkyl, haloalkoxy, heteroalkyl, -COR (where R is hydrogen, alkyl, phenyl or phenylalkyl, $-(CR'R'')_n$-COOR (where n is an integer from 0 to 5, R' and R" are independently hydrogen or alkyl, and R is hydrogen, alkyl, cycloalkyl, cycloalkyl-alkyl, phenyl or phenylalkyl), or -(CR'R")n-$CONR^xR^y$ (where n is

an integer from 0 to 5, R' and R" are independently hydrogen or alkyl, and $R^x$ and $R^y$ are, independently of each other, hydrogen, alkyl, cycloalkyl, cycloalkyl-alkyl, phenyl or phenylalkyl). In one embodiment, $R^x$ and $R^y$ together is cycloalkyl or heterocyclyl. More specifically the term heteroaryl includes, but is not limited to, pyridyl, furanyl, thienyl, thiazolyl, isothiazolyl, triazolyl, imidazolyl, isoxazolyl, pyrrolyl, pyrazolyl, pyridazinyl, pyrimidinyl, benzofuranyl, tetrahydrobenzofuranyl, isobenzofuranyl, benzothiazolyl, benzoisothiazolyl, benzotriazolyl, indolyl, isoindolyl, benzoxazolyl, quinolyl, tetrahydroquinolinyl, isoquinolyl, benzimidazolyl, benzisoxazolyl or benzothienyl, indazolyl, pyrrolopyrymidinyl, indolizinyl, pyrazolopyridinyl, triazolopyridinyl, pyrazolopyrimidinyl, triazolopyrimidinyl, pyrrolotriazinyl, pyrazolotriazinyl, triazolotriazinyl, pyrazolotetrazinyl, hexaaza-indenly, and heptaaza-indenyl and the derivatives thereof. Unless indicated otherwise, the arrangement of the hetero atoms within the ring can be any arrangement allowed by the bonding characteristics of the constituent ring atoms.

**[0024]** "Heterocyclyl" or "cycloheteroalkyl" means a saturated or unsaturated non-aromatic cyclic radical of 3 to 8 ring atoms in which one to four ring atoms are heteroatoms selected from O, NR (where R is hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, phenyl or phenylalkyl), $P(=O)OR^w$, or $S(O)_p$ (where p is an integer from 0 to 2), the remaining ring atoms being C, where one or two C atoms can optionally be replaced by a carbonyl group. The heterocyclyl ring can be optionally substituted independently with one, two, three or four substituents selected from alkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl, cycloalkylalkyl, halo, nitro, cyano, hydroxy, alkoxy, amino, mono-alkylamino, di-alkylamino, haloalkyl, haloalkoxy, -COR (where R is hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, phenyl or phenylalkyl), $-(CR'R")_n$-COOR (n is an integer from 0 to 5, R' and R" are independently hydrogen or alkyl, and R is hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, phenyl or phenylalkyl), or $-(CR'R")_n$-$CONR^xR^y$ (where n is an integer from 0 to 5, R' and R" are independently hydrogen or alkyl, $R^x$ and $R^y$ are, independently of each other, hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, phenyl or phenylalkyl). More specifically the term heterocyclyl includes, but is not limited to, pyridyl, tetrahydropyranyl, N-methylpiperidin-3-yl, N-methylpyrrolidin-3-yl, 2-pyrrolidon-1-yl, furyl, quinolyl, thienyl, benzothienyl, pyrrolidinyl, piperidinyl, morpholinyl, pyrrolidinyl, tetrahydrofuranyl, tetrahydrothiofuranyl, 1,1-dioxo-hexahydro-$1\Delta^6$-thiopyran-4-yl,

tetrahydroimidazo [4,5-c] pyridinyl, imidazolinyl, piperazinyl, and piperidin-2-only and the derivatives thereof. The prefix indicating the number of carbon atoms (*e.g.*, $C_3$-$C_{10}$) refers to the total number of carbon atoms in the portion of the cycloheteroalkyl or heterocyclyl group exclusive of the number of heteroatoms.

**[0025]** As used herein, "preventing" and grammatical equivalents thereof mean to reduce the risk of occurrence of cancer in an individual or in individuals in a population. That is, administration of an agent to a population of individuals "prevents" a condition when, relative to a control population, fewer individuals in the population develop the condition, and/or appearance of the condition is delayed in the administered population relative to a control population. Methods for detecting and quantifying a reduction of risk, including use of clinical trials, are well known in the art. As used herein, "preventing" a condition or disease in a patient refers to taking steps to obtain beneficial or desired results, including clinical results.

**[0026]** As used herein, "administering" or "administration of" a drug to a subject (and grammatical equivalents of these phrases) includes both direct administration, including self-administration, and indirect administration, including the act of prescribing a drug. For example, as used herein, a physician who instructs a patient to self-administer a drug and/or provides a patient with a prescription for a drug is administering the drug to the patient.

**[0027]** As used herein, a "prophylactically effective amount" of a drug is an amount of a drug that, when administered to a subject, will have the intended prophylactic effect, e.g., preventing the onset of disease or symptoms, or reducing or delaying the likelihood of the onset of disease or symptoms. A "prophylactically effective amount" of a drug can also be an amount of a drug that, when administered to a subject, reduces the likelihood of recurrence of disease or symptoms, or delays the onset of disease or symptoms. The full prophylactic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a prophylactically effective amount may be administered in one or more administrations.

**[0028]** As used herein, "cancer" has its ordinary medical meaning and refers to a malignancy (including head, neck, prostate and breast cancers, leukemias and lymphomas) generally characterized by clonality, autonomy, anaplasia and metastasis (see Mendelsohn, 1991). Epithelial cancer is any malignant neoplasm

originating from epithelium, *i.e.*, a carcinoma. Examples of epithelial cancer include prostate; breast; lung; bladder; colon; esophageal; pancreatic; head and neck; skin, including basal cell carcinoma and squamous cell carcinoma; and stomach cancer.

**[0029]** As used herein, "biopsy" refers to obtaining tissue from a patient and includes needle biopsy, resection and the like.

**[0030]** Abbreviations: The following abbreviations and acronyms are used: BPH, Benign prostatic hyperplasia (also called benign prostatic hypertrophy); LND, Lonidamine; LNDA, Lonidamine analog; LUTS, Lower Urinary Tract Symptoms, usually associated with benign prostatic hypertrophy (BPH); LND/A; the acronym "LND/A" is used for ease of reading and means "lonidamine, or alternatively a lonidamine analog, or alternatively either lonidamine or a lonidamine analog."

## 2. INTRODUCTION

**[0031]** Lonidamine is an agent that can disrupt energy metabolism in a cell. As noted above, lonidamine was approved in Italy and Portugal for the treatment of cancer, including lung, breast, prostate, and brain cancers, and the use of lonidamine and lonidamine analogs (LND/A) for treatment and prevention of benign prostatic hyperplasia has been described (see PCT patent publication Nos. 2004/064735 and 2004/064736, both incorporated by reference herein). The present invention relates to prevention of prostate cancer or other epithelial cell cancers using lonidamine, lonidamine analogs, and other agents (energolytic agents) that disrupt energy metabolism.

**[0032]** In one aspect, the invention relates to prevention of prostate cancer in a subject susceptible to developing prostate cancer (*i.e.*, a subject in a "prostate cancer susceptibility population") by administering lonidamine, a lonidamine analog or another energolytic agent.

**[0033]** In another aspect, the invention relates to prevention of an epithelial cancer other than prostate cancer (*e.g.*, breast cancer) by administering lonidamine, a lonidamine analog or another energolytic agent to a subject who will benefit from such administration, *i.e.*, a subject in an "epithelial cancer susceptibility population."

3. LONIDAMINE AND OTHER AGENTS

**[0034]** Lonidamine is 1-(2,4-dichlorobenzyl)-1-H-indazole-3-carboxylic acid. Lonidamine was first identified as having anti-spermatogenic activity, and subsequently used in a limited number of European countries for treatment of certain cancers. The use of lonidamine and lonidamine analogs for treatment and prevention of benign prostatic hyperplasia has been described. When lonidamine was administered for 28-days to men with BPH, subjects experienced an average decrease in prostate volume of greater than 10% (see Example 1, below, and Ditonno *et al.*, 2005).

**[0035]** Without intending to be bound by a particular theory, the effect of lonidamine on prostate tissue is believed due, at least in part, to killing of prostate epithelial cells. A brief discussion of prostate biology will aid in the understanding this effect. The prostate gland contains secretory epithelial cells in a stroma of connective tissue and smooth muscle (Barry *et al., 2*003). The secretory epithelial component in the normal prostate is remarkable in that the level of zinc in this tissue is exceedingly high compared to other normal tissues. A consequence of the high zinc level is that, through a mechanism involving zinc inhibition of the enzyme m-aconitase (a key enzyme in the tricarboxylic acid (TCA) cycle), the generation of energy via the TCA cycle and oxidative phosphorylation is substantially reduced in the secretory epithelium of the prostate, making this tissue far more dependent than other tissues and organs upon glycolysis as an energy source. Thus, prostate epithelial cells are uniquely dependent on glycolysis (anaerobic metabolism) and so uniquely susceptible to inhibitors of glycolysis and to agents that increase the near complete blockade of the TCA cycle in those cells. Another physiological result of the zinc-based inhibition of m-aconitase is the diversion of citrate from the TCA cycle, enabling the prostate to secrete large quantities of citrate, used by the sperm as an energy source and buffer, into the seminal fluid. See, *e.g.*, Costello and Franklin, 1997, 2000; Costello *et al.*, 1999, 2000.

**[0036]** Without intending to be bound by a specific mechanism, it is believed that administration of agents that inhibit glycolysis and/or mitochondrial function in prostate epithelial cells results in apoptosis of these cells. There are several lines of evidence that support this hypothesis. These include the following.

- Administration of lonidamine to men with BPH reduces serum prostate specific antigen (PSA) levels. See Example 1, *infra*. This is consistent with destruction of lonidamine-sensitive cells in the prostate.
- Administration of lonidamine to men with BPH reduces prostate size. See Example 1, *infra*.
- Lonidamine induces apoptosis in primary cultures of human prostate epithelial cells (see Example 2 and US patent application publication no. 2004/0167196 published August 26, 2004 and incorporated by reference herein.
- Lonidamine induces apoptosis in a cell line (LNCaP) derived from human prostate cells (see Example 2 and US 2004/0167196).

**[0037]** Without intending to be bound by a specific mechanism, it is believed that the methods of the invention provide for the preferential destruction of the citrate-producing cells in the prostate by inhibiting glycolysis or by further impairing mitochondrial function, or both, in those cells, such that enough of the citrate-producing cells are destroyed to reduce substantially the incidence of prostate cancer.

**[0038]** Thus, in accordance with the methods of the invention, an agent that inhibits or impairs energy production in prostate epithelial cells is administered to a human or other mammal susceptible to prostate cancer at a dose that impairs energy production (decreases ATP levels) for a period of time that results in the preferential destruction of at least some of the citrate producing cells by starving them, relative to the normal cells in the body, of energy. In some embodiments the agent is lonidamine. In some embodiments, the agent is a lonidamine analog, examples of which are described below in Section 7. In some embodiments, the agent is an agent other than lonidamine or a lonidamine analog that inhibits or impairs energy production in epithelial cells (i.e., an energolytic agent). Exemplary energolytic agents are described below in Section 8. Although for the sake of ease of reading the specification generally refers to lonidamine, it will be appreciated that the disclosure herein that relates to the uses of lonidamine apply *mutatis mutandis* to the uses of lonidamine analogs and energolytic agents. Some agents useful in the practice of the invention are identified by their ability to mimic one or more other activities of lonidamine, such as induction of apoptosis or inhibition of hypoxic induction of HIF-1alpha protein expression/accumulation in prostate epithelial cells or

cell lines *in vitro* (e.g., inhibition of hypoxic induction of HIF-1alpha protein expression/accumulation).

4. THE PROSTATE CANCER SUSCEPTIBILITY POPULATION

**[0039]** In one aspect of the invention, lonidamine, a lonidamine analog, or an energolytic agent is administered to a subject in need of prostate cancer prevention. As used herein, "a subject in need of prostate cancer prevention" is a man at risk for developing prostate cancer, e.g., a man diagnosed as at risk for developing prostate cancer. Prostate cancer risk can be determined using art-known methods and criteria.

**[0040]** In one aspect, the invention relates to prevention of prostate cancer by administering LND/A to a subject in a specified "prostate cancer susceptibility population." Prostate cancer susceptibility populations can be defined by both inclusionary and exclusionary criteria. All contemplated prostate cancer susceptibility populations exclude females, males less than 21 years old, and males currently or previously diagnosed with or under treatment for prostate cancer (as defined herein).

**[0041]** Certain particular a prostate cancer susceptibility populations may also exclude any male:

- diagnosed as having a cancer other than prostate cancer; and/or
- diagnosed with BPH; and/or
- under treatment for BPH; and/or
- diagnosed with LUTS (as defined herein); and/or
- under treatment for LUTS; and/or
- who has been prostatectomized.

In one embodiment a man with *any* of the above attributes is excluded from the prostate cancer susceptibility population. However, it will be appreciated that each of the above exclusionary criteria can be independently considered, alone or in combination with inclusionary criteria discussed below, in defining a particular (*i.e.*, different) prostate cancer susceptibility population.

**[0042]** In one aspect, the invention provides methods for prevention of prostate cancer in a man in a prostate cancer susceptibility population defined as above (e.g., not previously diagnosed with or under treatment for prostate cancer as defined

herein and optionally not diagnosed as having a cancer other than prostate cancer and/or diagnosed with or under treatment for BPH of LUTS) and further characterized as having an indicator associated with an increased likelihood of developing prostate cancer. Since the likelihood of developing prostate cancer increases with age, increased age can be used as an indicator. More often, an indicator other than age known to be associated with an increased likelihood of developing prostate cancer is used. Such non-age indicators include, without limitation, abnormal findings from a digital rectal examination, diagnosis of prostatic intraepithelial neoplasia (PIN), the presence of markers such as elevated and/or rising prostate specific antigen (PSA) levels or other prostate cancer-susceptibility markers, and a genetic predisposition to developing prostate cancer and/or familial history of prostate cancer, as described in more detail below.

**[0043]** In an embodiment, the invention provides a method of treating a human subject to reduce the likelihood of developing prostate cancer comprising (a) identifying the subject as being in a prostate cancer susceptibility population, and (b) administering a prophylactically effective amount of lonidamine (LND) or a lonidamine analog (LNDA) or energolytic agent to the subject.

**[0044]** Aspects of each of the inclusionary and exclusionary criteria are discussed below.

A) Exclusionary Criteria Used In Certain Embodiments

i. Prostate Cancer

**[0045]** Men "diagnosed as having prostate cancer" refers to a positive clinical diagnosis that a subject has prostate cancer, i.e., a diagnosis confirmed by analysis of prostate tissue (e.g., obtained by biopsy). It will be appreciated that prostate cancer is a slow growing cancer. Cancers are often clonal, indicating that a single prostate cancer cell may appear the prostate years before cancer can be detected or diagnosed in the clinic. However, a man with a single prostate cancer cell is not considered to have prostate cancer as the term is used herein. Indeed, a man with hundreds or even thousands of prostate cancer cells is not considered to have prostate cancer as the term is used herein if the cancer cannot be detected or positively diagnosed using currently used and generally accepted methods for diagnosing prostate cancer. Methods for diagnosis of prostate cancer are known

and can include assessment of multiple factors including abnormal findings from a digital rectal examination, hypoechoic lesions detected by transrectal ultrasound (TRUS), serum PSA levels indicative of cancer, and/or histologically detected abnormality. In one embodiment a diagnosis of prostate cancer as used herein requires the presence of indicators sufficient to establish basis for currently approved treatments (e.g., chemotherapy, radiation therapy or prostatectomy). In one embodiment a diagnosis of prostate cancer as used herein requires histological evidence (i.e., analysis of tissue obtained by biopsy) of cancer. In one embodiment a diagnosis of prostate cancer as used herein requires non-histological evidence (e.g., results from an assay for a cancer marker) that is accepted as a surrogate of histological results. A result from a non-histological assay may be considered a surrogate of histological evidence of cancer when a positive result in the non-histological assay has an at least 90% correlation, and alternatively an at least 95% correlation, with a positive histological indication of cancer. In one embodiment, the "a subject in need of prostate cancer prevention" is a man who has undergone a prostate biopsy in which no evidence of prostate cancer was detected.

ii. Cancer Other Than Prostate Cancer

**[0046]** In some embodiments of the invention, men who have been diagnosed as having a cancer (other than prostate cancer) and/or are under treatment for a cancer (other than prostate cancer) are not included in the prostate cancer susceptibility population.

iii. Benign Prostatic Hyperplasia (BPH)

**[0047]** In some embodiments of the invention, men who have been diagnosed as having BPH and/or are under treatment for BPH are not included in the prostate cancer susceptibility population. BPH can be diagnosed using methods known to physicians (see, The Merck Manual of Diagnosis and Therapy, Section 17. Genitourinary Disorders Chapter 218. Prostate Disease). The most common test is the digital rectal examination in which a physician determines whether the prostate is of a normal size and firmness. Other diagnostic assays include a urine flow rate test, determination of post void residual urine volume (*e.g.*, by palpitation of the abdomen, drainage of residual urine, x-ray urogramography, or ultrasonography), moderate or

severe symptom scores on the American Urologic Association Symptom Index (AUASI; Barry *et al.*, 1992) or International Prostate Symptom Score (IPSS; Barry *et al.*, 2001).

**[0048]** In one embodiment lonidamine is administered to a man not under treatment for BPH. BPH is treated using procedures such as surgery (transurethral resection of the prostate; transurethral incision of the prostate; or open prostatectomy), laser therapy, transurethral microwave thermotherapy, balloon dilatation, placement of a prostatic urethral stent, transurethral needle ablation, and transurethral electrovaporization of the prostate or by administration of drugs such as alpha-adrenergic-blockers (e.g., doxazosin, terazosin, tamsulosin, alfuzosin, and prazosin), 5-alpha-reductase (e.g., finasteride), and lonidamine (see US 2004/0167196). Accordingly, subjects predicted to benefit significantly from the methods of the invention can be selected in a population of men with BPH by identifying subjects with a serum PSA value greater than 2 ng/ml. In one embodiment of the invention, the subject has a PSA level greater than about 4 ng/ml. Because higher PSA levels are closely associated with prostate cancer, in one embodiment, the subject selected for therapy with an energolytic agent has a PSA level greater than about 10 ng/ml.

iv. Lower Urinary Tract Symptoms (LUTS) Associated With BPH

**[0049]** In some embodiments of the invention, men diagnosed as having, or under treatment for, LUTS (as defined herein) are not included in the prostate cancer susceptibility population. LUTS is an acronym for Lower Urinary Tract Symptoms, which are usually associated with benign prostatic hypertrophy (BPH). Thus, in certain embodiments of the invention, the prostate cancer susceptibility population does not include men diagnosed as having and under treatment for one or more of the following symptoms: (1) urinary urgency; (2) terminal dribbling of urine; (3) frequent urination; (4) nocturia; (5) a weak/slow stream of urine; (6) a sense of incomplete emptying; (7) intermittency; (8) straining; (9) dysuria; (10) hematuria; (11) acute urinary retention; (12) urinary tract infection; and (13) incontinence.

v. Prostatectomy

**[0050]** In some embodiments of the invention, men who have had surgical prostatectomy are not included in the prostate cancer susceptibility population.

B) Inclusionary Criteria (Indicators) Used In Certain Embodiments

**[0051]** As noted above, in one aspect of the invention, lonidamine, a lonidamine analog, or an energolytic agent is administered to a subject in need of prostate cancer prevention such as a subject in a specified prostate cancer susceptibility population described herein. In various embodiments the subject may be diagnosed as having Prostatic Intraepithelial Neoplasia (PIN) and/or having one or more (i.e., at least one) non-age indicators associated with an increased likelihood of developing prostate cancer and/or being at an increased likelihood of developing prostate cancer due to age.

i) Prostatic Intraepithelial Neoplasia

**[0052]** In one aspect, the invention provides a method for preventing prostate cancer in a human patient by administering a therapeutically effective amount of lonidamine, a lonidamine agent or an energolytic agent to the patient. In an embodiment, an agent is administered to a subject diagnosed as having Prostatic Intraepithelial Neoplasia (PIN). PIN is characterized by abnormal cellular proliferation within the prostatic ducts, ductules and acini. PIN has previously been referred to as "intraductal hyperplasia," "hyperplasia with malignant change," "large acinar atypical hyperplasia," "marked atypia" and "ductal-acinar dysplasia." A subject diagnosed as having PIN is, in one embodiment of the invention, at increased risk of developing prostate cancer. PIN is sometimes characterized as high grade PIN (HGPIN) or low grade PIN. HGPIN is associated with the progressive development of abnormalities in the normal prostatic epithelium, leading to a cancerous condition. See, e.g., Bostwick, 1992. Patients diagnosed as having HGPIN have an increased likelihood of developing prostate cancer within 10 years.

**[0053]** In one embodiment, the invention provides a method for preventing prostate cancer in a human patient diagnosed with High-Grade Prostatic Intraepithelial Neoplasia (HGPIN) by administering a therapeutically effective amount of lonidamine, a lonidamine agent or an energolytic agent to the patient. HGPIN is

characterized by marked cellular proliferation within prostatic ducts, ductules, and acini. For example, HGPIN is associated with proliferative changes within preexisting acini, nuclear and nucleolar abnormalities (e.g., nuclear enlargement and hyperchromasia, nuclear overlapping or pseudo-stratification, prominent nucleoli), and fragmentation of basal cell layer. Typical architectural patterns are associated with HGPIN are tufting, micropapillary, cribiform and flat. See, e.g., Bostwick *et al.*, 1993. Other, less common patterns of HGPIN include a signet ring-cell pattern, a small cell neuroendocrine pattern, and a mucinous pattern. Proliferation associated with HGPIN can spread through the prostatic ducts in different patterns. In a first pattern, neoplastic cells replace the normal luminal secretory epithelium, but the basal layer and basement membrane are preserved. A second pattern is characterized by direct invasion through the ductal or acinar wall with disruption of the basal cell layer. In a third, relatively rare pattern, neoplastic cells invaginate between the basal cell layers, sometimes described as pagetoid spread. In accordance with the practice of the invention, in one embodiment an agent, e.g., lonidamine, is administered to a patient having HGPIN characterized by the first spreading pattern. In other embodiments, an agent is administered to a patient having HGPIN characterized by the second or third spreading pattern.

**[0054]** In another embodiment, the patient is diagnosed with Low Grade Prostatic Intraepithelial Neoplasia (LGPIN). In LGPIN, the epithelium lining ducts and acini are heaped up, crowded and irregular. See, e.g., Bostwick, 1992. Elongated, hyperchromatic nuclei and small nucleoli can be present. Lesions displaying some, but not all features, are considered atypical, but may not be neoplastic. LGPIN is present in many men by the third decade of life. Patients with LGPIN can be monitored for progression to HGPIN.

**[0055]** PIN can be diagnosed by any method known to the skilled artisan and accepted in the medical community. For example, PIN can be diagnosed by needle biopsy, such as a transrectal ultrasound-guided biopsy. See, e.g., Borboroglu *et al.*, 2000; Davidson *et al.*, 1995. Prostate biopsies are often performed due to a presence of a palpable lesion in the prostate or other indicia know to physicians. Typically, 8-10 cores are prepared for examination by a pathologist for characteristic morphological changes in the prostate. If evidence of PIN is detected, PIN can be monitored, for example, by monitoring PSA levels, as elevated PSA levels may

indicate progression to a cancerous state. If the alterations associated with PIN continue, a progressive loss of some markers of secretory differentiation occurs. Such markers include secretory proteins, cytoskeletal proteins, glycoproteins, and neuroendocrine cells. There can also be a progressive increase in c-erb-b2 oncoprotein, bcl-2, epidermal growth factor, epidermal growth factor receptor, type 4 collagenase, Lewis Y antigen, transforming growth factor–alpha, apoptotic bodies, proliferating cell nuclear antigen expression, aneuploidy and a variety of genetic abnormalities, and/or microvessel density. PIN diagnosis also can be performed on biopsy samples by, for example, measuring uteroglobin protein or mRNA levels. See, e.g., U.S. patent publication No. 2002/0151470, published October 17, 2002 (the disclosure of which is incorporated by reference herein).

**[0056]** According to another example, increased likelihood of having PIN can be determined by assaying for Prostate Stem Cell Antigen (PSCA) positive (+) cells within various biological samples, such as serum and prostate biopsy specimens. See, e.g., U.S. patent publication No. 2005/0059099, published March 17, 2005 (the disclosure of which is incorporated by reference herein). Optionally, morphological analysis can be used to confirm a diagnosis of a grade of PIN.

**[0057]** The efficacy of a particular energolytic agent, dose, or administration regimen in the practice of the present invention, can be established in a variety of ways. In one embodiment, a subject diagosed with PIN and treated with Ionidamine, a Ionidamine analog or another energolytic agent is evaluated after a period of treatment (e.g., 1 month, six months, one year, etc.) or number of drug administrations (e.g., one administration, daily administration for 1 to 6 months, etc.) and a needle biopsy of the subject's prostate is preformed and evaluated for evidence of PIN. The absence of indicators of PIN or a reduction of the extent of PIN is indicative of the efficacy of the agent, dose, or administration regimen in preventing prostate cancer (or, equivalently, treating PIN) in the individual.

**[0058]** In an alternative embodiment, a clinical trial is used to assess the efficacy of a particular energolytic agent, dose, or administration regimen in the practice of the present invention. For example, patients diagnosed with HGPIN can be enrolled and receive the agent (e.g., Ionidamine) or a placebo, and subjected to one or more follow-on biopsies at a later time point(s) (e.g., six months, one year and five years the initiation of therapy). An efficacy parameter would be a reduction in the rate of

detection of prostate cancer in the agent population compared to the placebo population. For example, the rate might be reduced by at least 20%, at least 30% or more.

ii) Abnormal Results from a Digital Rectal Examination or Imaging Methods

**[0059]** In one embodiment, an agent is administered to a subject not diagnosed with prostate cancer, but instead diagnosed by digital rectal examination as having an abnormal prostate size or shape (e.g., a palpable lump). Prostate size and shape can be used to assess prostate cancer risk; the most common test for prostate size and shape is the digital rectal examination in which a physician determines whether the prostate is of a normal size and firmness. Abnormalaties of size, shape or structure (e.g., density) can also be detected using imaging methods such as ultrasound and MRI.

iii. Prostate Specific Antigen Levels

**[0060]** In one embodiment, an agent is administered to a subject not diagnosed with prostate cancer, but having an elevated serum prostate specific antigen (PSA) level. Men 40 years old or younger typically have blood PSA levels below 2.0 ng/ml. However, the range of "normal" PSA increases with age. Normal PSA levels will vary according to the size of the prostate, race, and assay method as well as age, but typical values are shown in Table 1.

Table 1

| Age | PSA Level |
|---|---|
| 40 and younger | 0 to 2.0 ng/mL |
| 45 | 0 to 2.4 ng/mL |
| 50 | 0 to 2.8 ng/mL |
| 55 | 0 to 3.3 ng/mL |
| 60 | 0 to 3.8 ng/mL |
| 65 | 0 to 4.5 ng/mL |
| 70 | 0 to 5.3 ng/mL |
| 75 | 0 to 6.2 ng/mL |
| 80 and older | 0 to 7.2 ng/mL |

**[0061]** In one embodiment of the invention, the subject has a higher than normal PSA level. In one embodiment of the invention, the subject has a PSA level greater than about 2 ng/ml. In one embodiment of the invention, the subject has a PSA level greater than about 2 ng/ml but less than about 8 ng/ml. In one embodiment of the invention, the subject has a PSA level greater than about 2 ng/ml but less than about 10 ng/ml. In one embodiment of the invention, the subject has a PSA level from 0 to 9 ng/ml, 0 to 8 ng/ml, 0 to 7 ng/ml, 0 to 6 ng/ml, 0 to 5 ng/ml, 0 to 4 ng/ml, 0 to 3 ng/ml or less than 2 ng/ml. In one embodiment of the invention, the subject has a PSA level greater than about 4 ng/ml. In one embodiment of the invention, the subject has a PSA level greater than about 4 ng/ml but less than about 10 ng/ml.

iv. Rising PSA Levels

**[0062]** In one embodiment, an agent is administered to a subject not diagnosed with prostate cancer, but having a rising PSA level. Increases in PSA levels over time are also correlated with prostate cancer. Subjects predicted to benefit significantly from the methods of the invention can be selected in a population of men with rising serum PSA levels. In one embodiment, the phrase "rising PSA levels" means a velocity of at least 0.50 ng/ml/yr or at least 0.75 ng/ml/yr. In one embodiment, the phrase "rising PSA levels" means a PSA doubling time of less than 120 months. PSA levels can be determined as discussed above.

v. Prostate Cancer-Susceptibility Markers

**[0063]** In one embodiment, an agent is administered to a subject not diagnosed with prostate cancer, but is identified as having an elevated level of a prostate-cancer susceptibility marker. Such markers can include, for example, prostatic acid phosphatase (Fong et al., 2003); AMACR (Evans, 2003); prostate-specific membrane antigen (PSMA); Pro109 (Freje et al., 1993); Pro112 WO9514772 and WO9845436); Pro111 (Dubbink et al., 1998); Pro115 (Paoloni-Giacobino et al., 1997; WO9837418 and WO987093); Pro110 (U.S. Patent No. 5,665,874 and PCT publication WO9403599); Pro113 (Steinicki et al., 1998); Pro114 (WO9839446); and Pro118 (WO9845435). See also U.S. Patent No. 6,902,892. Methods for detecting expression of such markers are described (supra) or known in the art. Although such markers have been reported to be associated with the presence of prostate cancer,

the expression or over-expression of such markers does not provide a positive diagnosis of the presence of prostate cancer.

vi. Genetic Predisposition to Developing Prostate Cancer

**[0064]** In one embodiment, an agent is administered to a subject not diagnosed with prostate cancer, but who instead has been diagnosed as having a genetic predisposition to developing prostate cancer. Prostate cancer risk can also be assessed by screening of genetic markers associated with an increased risk of developing prostate cancer. For example, certain polymorphisms of the CAPB, HPC1/RNASEL, HPC2/ELAC2, HPCX, MSR1, PCAP, HPC20, SRD5A2, androgen receptor (AR) and VDR loci are associated with increased susceptibility to prostate cancer. See Nieder *et al.*, 2003. See also Verhage *et al.*, 2003.

**[0065]** In one embodiment, a subject in a prostate cancer susceptibility population has polymorphism in at least one of the CAPB, HPC1/RNASEL, HPC2/ELAC2, HPCX, MSR1, PCAP, HPC20, AR, SRD5A2 and VDR loci, which polymorphism is associated with an increased risk of developing prostate cancer. Methods of detecting polymorphisms in susceptibility loci are known to persons of skill in the art. Such methods can include, for example, polymerase chain reaction-based methods, restriction fragment length polymorphism analysis (RFLP) and single strand conformation analysis (SSCP). Methods of differentiating polymorphic (allelic) variants utilize molecular marker techniques well known to those of skill in the art including such techniques as: 1) single stranded conformation analysis (SSCP); 2) denaturing gradient gel electrophoresis (DGGE); 3) RNase protection assays; 4) allele-specific oligonucleotides (ASOs); 5) the use of proteins which recognize nucleotide mismatches, such as the *E. coli* mutS protein; and 6) allele-specific PCR. See generally Ausubel *et al.*, Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y. (1998) including supplements to 2004. *See* Dunning *et al.*, 1999.

vii. Familial History of Prostate Cancer

**[0066]** In one embodiment, an agent is administered to a subject not diagnosed with prostate cancer, but having a familial history of developing prostate cancer. A risk of developing prostate cancer can be correlated with a familial history of prostate

cancer. The increased risk has been reported to be two- to three-fold greater. See Verhage *et al.*, 2003. The risk is higher having multiple affected relatives or having relatives diagnosed at an early age with prostate cancer. *Id.*

**[0067]** Such a risk can be determined, for example, from a family history. For example, subjects having a cluster of three or more first-degree relatives with prostate cancer can have an increased risk of developing prostate cancer. See Nieder *et al.*, 2003. Subjects having a family history of prostate cancer in each of three generations in the paternal or maternal lineage also can have an increased risk of developing prostate cancer. *Id.* Also, subjects having two or more first- or second-degree relatives with prostate cancer under age 55 have an increased risk of developing prostate cancer. *Id.*

**[0068]** Thus, in one embodiment, a subject in a prostate cancer susceptibility population has a cluster of three or more first-degree relatives with prostate cancer can have an increased risk of developing prostate cancer. In another embodiment, a subject in a prostate cancer susceptibility population has a family history of prostate cancer in each of three generations in the paternal or maternal lineage. In another embodiment, a subject in a prostate cancer susceptibility population has two or more first- or second-degree relatives under age 55 diagnosed with prostate cancer. In another embodiment, a subject in a prostate cancer susceptibility population has at least one male relative selected from the group consisting of sons, father, and uncles who has had prostate cancer.

viii) Increased Risk of Developing Prostate Cancer Due to Age and Other

**[0069]** Age is a risk factor for developing prostate cancer, with more than 75% percent of prostate cancer diagnosed in men ages 65 or older. In various embodiments of the present invention, the prostate cancer susceptibility population (i) comprises men 40 years or older (i.e., excludes men younger than 40 years old); (ii) comprises men 50 years or older (i.e., excludes men younger than 50 years old) or (iii) comprises men 60 years or older (i.e., excludes men younger than 60 years old). In various other embodiments the prostate cancer susceptibility population (i) comprises men younger than 40 years (i.e., excludes men 40 years or older); (ii) comprises men younger than 50 years (i.e., excludes men 50 years or older); (iii) comprises men younger than 60 years (i.e., excludes men 60 years or older).

**[0070]** In one embodiment lonidamine is administered to a man BPH identified as having a serum PSA value greater than about 10 ng/ml.

xi. Exemplary Prostate Cancer Susceptibility Populations

**[0071]** In an embodiment, the invention provides a method of treating a human subject to reduce the likelihood of developing prostate cancer comprising (a) identifying the subject as being in a prostate cancer susceptibility population, and (b) administering a prophylactically effective amount of lonidamine (LND) or a lonidamine analog (LNDA) or energolytic agent to the subject. As discussed above, some prostate cancer susceptibility populations can be defined by both exclusionary and inclusionary criteria. For illustration and not limitation exemplary prostate cancer susceptibility populations who would benefit from prevention of prostate cancer include any of the following populations

1. Prostate Cancer- No; Other Cancer- No; PIN- Yes; BPH- No; LUTS (any symptom) -No
2. Prostate Cancer- No; Other Cancer- No; PIN- Yes; BPH- Yes; LUTS (any symptom) -Yes
3. Prostate Cancer- No; Other Cancer- Yes; PIN- Yes; BPH- No; LUTS (any symptom) -No
4. Prostate Cancer- No; Other Cancer- No; PIN- No; BPH- No; LUTS (any symptom) -No; Age- 50
5. Prostate Cancer- No; Other Cancer- No; PIN- No; BPH- No; LUTS (any symptom) -No; Non-age indicator (e.g. abnormal findings from DRE; higher than normal PSA; other prostate cancer-susceptibility markers; genetic predisposition to developing prostate cancer; familial history of prostate cancer) - Yes

5. EPITHELIAL CANCER SUSCEPTIBILITY POPULATION

**[0072]** In another aspect of the invention, methods for preventing epithelial cancer in a subject are provided. Without intending to be bound by a particular theory, the effect of lonidamine on cancerous or precancerous epithelial cells is believed due, at least in part, to killing of such epithelial cells. Without intending to be bound by a specific mechanism, it is believed that administration of agents (e.g., lonidamine, a lonidamine analog or an energolytic agent) that inhibit glycolysis and/or mitochondrial function in precancerous and cancerous epithelial cells results in apoptosis of these cells. This hypothesis is supported by animal studies in which prophylactic administration of lonidamine to rats reduced the incidence of a spontaneous epithelial cell cancer, breast cancer (see Example 3, *infra*).

**[0073]** In one embodiment, the invention provides a method of prophylaxis of an epithelial cell cancer other than prostate cancer comprising administering a prophylactically effective amount of lonidamine or a lonidamine analog or an energolytic agent to the subject in need of such prophylaxis.

**[0074]** In a related embodiment, the invention provides a method of treating a human subject to reduce the likelihood of developing an epithelial cancer, said method comprising (a) identifying the subject as being in an "epithelial cancer susceptibility population," and (b) administering a prophylactically effective amount of lonidamine or a lonidamine analog to the subject. Patients in the epithelial cancer susceptibility population do not have a diagnosed epithelial cancer but may have an indicator associated with an increased likelihood of developing an epithelial cancer. Epithelial cancer susceptibility populations can be defined by both inclusionary and exclusionary criteria.

A) Exclusionary Criteria

**[0075]** Thus, the epithelial cancer susceptibility population excludes patients:

- diagnosed as having an epithelial cancer (a malignant neoplasm originating from epithelium, i.e., a carcinoma, e.g., prostate; breast; lung; bladder; colon; esophagus; pancreas; head and neck; skin or stomach cancer);
- diagnosed as having a cancer other than an epithelial cancer;
- previously diagnosed as having epithelial cancer; and/or
- with BPH.

**[0076]** Epithelial cancer is typically indicated by abnormal physical examination results (e.g., abnormal breast examination results), by abnormal results from an X-ray, ultrasonographic or other procedure. A positive diagnosis usually involves histologic confirmation, such as by biopsy or other diagnostic measure. As used herein, “diagnosed as having epithelial cancer” refers to a positive clinical diagnosis that a subject has epithelial cancer, and can include a diagnosis confirmed by analysis of tissue (e.g., obtained by biopsy).

B) Inclusionary Criteria (Indicators)

**[0077]** Inclusionary criteria for an epithelial cancer susceptibility population are indicators associated with an increased likelihood of developing epithelial cancer

such as, without limitation, a genetic predisposition to developing epithelial cancer and/or familial history of epithelial cancer.

**[0078]** A subject having increased risk of developing epithelial cancer has one or more (i.e., at least one) indicators associated with an increased likelihood of developing epithelial cancer. Such indicators include those set forth below.

*Epithelial Cancer-Susceptibility Markers*

**[0079]** In one embodiment, an agent is administered to a subject not diagnosed with epithelial cancer, but instead diagnosed as having an elevated level of an epithelial cancer susceptibility marker. Such markers can include, for example, CA-125 (epithelial cancer), HER2 (breast cancer), Topoisomerase II alpha (ovarian epithelial cancer), Werner helicase interacting protein (ovarian epithelial cancer), HEXIM1 (ovarian epithelial cancer), FLJ20267 (ovarian epithelial cancer), Deadbox protein-5 (ovarian epithelial cancer), Kinesin-like 6 (ovarian epithelial cancer), p53 (ovarian epithelial cancer) and NY-ESO-1 (ovarian epithelial cancer). See, e.g., Menard *et al.*, 2004; WO 03/064593.

*Genetic Predisposition to Developing Epithelial Cancer*

**[0080]** In one embodiment, an agent is administered to a subject not diagnosed with epithelial cancer, but who instead has been diagnosed as having a genetic predisposition to developing epithelial cancer. Epithelial cancer risk can also be assessed by screening of genetic markers associated with an increased risk of developing epithelial cancer. For example, the BRCA1, BRCA2, p53, PTEN, ATM, NBS1 or LKB1 loci are associated with increased susceptibility to epithelial breast cancer. See, e.g., Dumitrescu *et al.*, 2005.

**[0081]** In one embodiment, a subject in an epithelial cancer susceptibility population has a polymorphism in at least one of the BRCA1, BRCA2, p53, PTEN, ATM, NBS1 and LKB1, which polymorphism is associated with an increased risk of developing epithelial cancer. In another embodiment, a subject in an epithelial cancer susceptibility population has a polymorphism at the BRCA1 locus associated with an increased risk of developing epithelial cancer. In another embodiment, a subject in an epithelial cancer susceptibility population has a polymorphism at the p53 locus associated with an increased risk of developing epithelial cancer. In

another embodiment, a subject in an epithelial cancer susceptibility population has a polymorphism at the PTEN locus associated with an increased risk of developing epithelial cancer. In another embodiment, a subject in an epithelial cancer susceptibility population has a polymorphism at the ATM locus associated with an increased risk of developing epithelial cancer. In another embodiment, a subject in an epithelial cancer susceptibility population has a polymorphism at the NBS1 locus associated with an increased risk of developing epithelial cancer. In another embodiment, a subject in an epithelial cancer susceptibility population has a polymorphism at the LKB1 locus associated with an increased risk of developing epithelial cancer. Methods of detecting polymorphisms in susceptibility loci are known to persons of skill in the art and include those described hereinabove.

*Familial History of Epithelial Cancer*

**[0082]** In one embodiment, an agent is administered to a subject not diagnosed with epithelial cancer, but diagnosed instead as having a familial history of developing epithelial cancer. A risk of developing epithelial cancer can be correlated with a familial history of epithelial cancer. See, e.g., Dumitrescu *et al.*, 2005 (breast cancer). The risk is higher for subjects having multiple affected relatives or having relatives diagnosed at an early age with epithelial cancer. Such a risk can be determined, for example, from a family history. For example, subjects having one or more first-degree relatives with epithelial cancer can have an increased risk of developing breast cancer. Thus, in one embodiment, a subject in an epithelial cancer susceptibility population has a cluster of one or more first-degree relatives with epithelial cancer can have an increased risk of developing epithelial cancer.

6. DOSE, ROUTE, SCHEDULE AND DURATION OF ADMINISTRATION

**[0083]** A variety of routes, dosage schedules, and dosage forms are appropriate for administration of agents according to the invention to prevent prostate or other epithelial cancer. Appropriate dosage schedules and modes of administration will be apparent to the ordinarily skilled practitioner upon reading the present disclosure and/or can be determined using routine pharmacological methods and/or methods described herein.

**[0084]** The dose, schedule and duration of administration of the agent will depend on a variety of factors. The primary factor, of course, is the choice of a specific agent. Other important factors include the age, weight and health of the subject, the severity of symptoms, if any, the subject's medical history, co-treatments, goal (e.g., prophylaxis or prevention of relapse), preferred mode of administration of the drug, the formulation used, patient response to the drug, and the like. Guidance concerning administration is provided by prior experience using the agent for a different indication (e.g., lonidamine administered to treat cancer is administered in 150 mg or 300 mg doses three times a day for a period of about a month) and from new studies in humans (e.g., lonidamine administered to treat BPH has been administered in 150 mg doses once a day for a period of about a month) and other mammals. Cell culture studies are frequently used in the art to optimize dosages, and the assays disclosed herein can be used in determining such doses (e.g., to determine the dose that induces significant apoptosis in prostate epithelial cells but not in other cells, such as, for example, liver cells). For particular agents, the scientific literature (including, for example, patent and non-patent publications cited herein) provides considerable guidance as to dosages, formulations and dosage forms for specific agents or classes of agents, *e.g.*, dosages known or predicted to result in a biologically effective serum level of the agent (or metabolite) in serum.

**[0085]** For example, an agent can be administered for the prevention of prostate cancer at a dose in the range of about 0.1 mg to about 100 mg of the agent per kg of body weight of the patient to be treated per day, optionally with more than one dose being administered per day, and typically with the daily dose being administered on multiple consecutive days. In one embodiment, an agent is administered in a dose in the range of about 0.2 mg to about 5 mg per kg of body weight of the patient to be treated per day. In another embodiment, an agent is administered in a dose in the range of about 0.2 mg to about 1 mg per kg of body weight of the patient to be treated. In certain other embodiments, an agent is administered in a dose of about 25 to 250 mg. In another embodiment, a prophylactically effective dose is about 25 to about 150 mg. For illustration, the prophylactically effective dose of an agent can be administered daily or once every other day or once a week to the patient. Controlled and sustained release formulations of the agents may be used. Generally, multiple administrations of the agent are employed. Depending on the

dose selected by the practitioner and the convenience of the patient, the entire dose may be administered once daily, or the dose may be administered in multiple smaller doses through the course of a day. For example, the dose may be divided into two smaller doses and administered twice daily, or divided into three smaller doses and administered thrice daily. Alternatively, the dose may be combined and given every other day, or even less frequently, but in any event, the dose is repeatedly administered over a period of time. For optimum treatment benefit, the administration of the prophylactically effective dose may be continued for multiple days, such as for at least five consecutive days, and often for at least a week and often for several weeks or more. In one embodiment, the agent is administered once (qday), twice (bid), three times (tid), or four times (qid) a day or once every other day (qod) or once a week (qweek), and treatment is continued for a period ranging from three days to two weeks or longer.

**[0086]** In the case of lonidamine, other exemplary dosage schedules are described in copending U.S. patent application no. 10/759,337, filed January 16, 2004, now U.S. patent publication No. US 20040167196, which is incorporated herein by reference. In one embodiment, the dosage form is the 150 mg unit dosage form marketed under the trade name DORIDAMINA™ (e.g., 150 mg po TID for about thirty days), and this dosage form is administered from once to three times daily for full preventive effect. Other dosing regimens contemplated include, for example and not for limitation, "low dosing" (e.g., dosages in the range of 1-300 mg per day total daily dosage, 5-300 mg/day, 5-70 mg/day, 1-25 mg/day, 20-45 mg/day, 40-65 mg/day, 40-70 mg/day, 50-100 mg/day, 50-200 mg/day, and 50-300 mg/day), "high dosing (e.g., total daily doses greater than 0.5 g, such as doses in the range 0.5 – 5 g/day, 0.5 – 3 g/day, 0.5 – 1 g/day and 1-3 g/day, or higher doses), and "intermediate dosing" (e.g., doses greater than 300 and less than 500 mg/day, such as doses in the range >300-400 or 400<500 , e.g., 450 mg/day).

**[0087]** For illustration and not limitation, the present invention also provides a pharmaceutical formulation of an energolytic agent suitable for oral administration (including tablets, capsules, and pills) and contains between 1 and 100 mg of the compound, and in another embodiment between 1 and 10 mg of the compound. In another embodiment, the formulation contains between 200 and 1000 mg of the compound, and in another embodiment between 500 and 1000 mg of the compound.

**[0088]** This treatment time period may include continuous dosing TID for two to six months or more or for only one to eight weeks. A dose of 150 mg po TID for 7-30 days of certain agents of the invention, such as lonidamine (and its analogs) can allow for the full therapeutic benefit for an extended period of time (3 months or longer, including up to 6 months to one year) while limiting or eliminating the unwanted side effects. In yet another embodiment, prophylactic treatment of prostate cancer in accordance with the methods of the invention by administering to a patient a much higher dose of an agent for a shorter period of time (that is, fewer administrations; in one embodiment, a single administration of an agent is sufficient to provide protection for a period of 3 to 6 months or longer).

**[0089]** In some embodiments, low doses are administered for prophylaxis. Exemplary very low dose regimens include (in mg) less than 25 per day, less than 20 per day, less than 15 per day, less than 10 per day, and less than 5 per day. Exemplary very low dose regimens include (in mg) less than 1 mg/month (administered by any treatment schedule, e.g. one 1 mg dose, 30 time 33 mg dose, 4 time 250 mg dose, etc.), less than 500 mg/month, less than 250 mg/month, or less than 100 mg/month. In one embodiment, a low dose form is used for a maintenance dose after a higher initial, priming or loading dose.

**[0090]** In these prophylactic applications, the agent can be administered a single time or many times over periods as long as several months or years. In one embodiment, the treatment is continued for one to three months. In another embodiment, the treatment is continued for a year. Thus, a patient may be administered the agent for a week, a month, two months, three months, six months, one year, two years, five years, ten years, twenty years or longer. For some applications, treatment may continue indefinitely throughout the life of the patient. As is well understood in medicine, treatment may be suspended temporarily if toxicity is observed or for the convenience of the patient without departing from the scope of the invention.

**[0091]** When formulated for oral delivery, preferred dosage forms include pills, tablets, capsules, caplets, and the like, optionally formulated for sustained release. Other suitable forms for oral administration include troches, elixirs, suspensions, syrups, wafers, lozenges, and the like. Other modes of administration are also contemplated, including parenteral, inhalation spray, transdermal, rectal,

intraprostetic injection (e.g., of agent-containing microparticles) and other routes. See, Ansel *et al.*, 1999; and Marshall, 1979.

**[0092]** In one embodiment, the dosage form is the 150 mg unit dosage form marketed for the treatment of cancer by ACRAF in Italy under the trade name DORIDAMINA™. DORIDAMINA™ contains 150 mg lonidamine, 87 mg starch, 55 mg microgranular cellulose, 40 mg lactose, 15 mg sodium carboxymethyl starch, 11 mg hydroxypropyl methylcellulose, 4.5 mg precipitated silica, 3.5 mg magnesium Stearate, 0.4 mg titanium dioxide, and 0.4 mg polyethylene glycol 400. Another formulation is 150 mg lonidamine (intra-granular), 87 mg cornstarch (intra-granular), 40 mg lactose (intra-granular), 3 mg colloidal silicon dioxide (intra-granular), 1.5 mg colloidal silicon dioxide (extra-granular), 55 mg microcrystalline cellulose (extra-granular), 15 mg sodium starch glycolate (extra-granular), 3.5 mg magnesium stearate (extra-granular), 9 mg hydroxypropyl methylcellulose (extra-granular), and 10.9 mg OPADRY white (coating).

**[0093]** It will be appreciated that these dosing schedules are for illustration and not limitation, and that a dosing schedule may change during a course of therapy based on, for example, a patient's response to the drug administered. As is well understood in the medicine, treatment may be suspended temporarily if toxicity is observed or for the convenience of the patient without departing from the scope of the invention.

**[0094]** For prevention of prostate cancer, results of prevention of prostate cancer in a subject may include a reduction in prostate size, a reduction in serum PSA, and/or results that will be recognized by a treating physician as indicative of a apoptosis of prostate cells (e.g., epithelial cells). In one aspect of the invention prostate size decreases by at least 10%, at least 20% or at least 40% and/or serum PSA levels decreases by at least 10%, at least 20% or at least about 40%, when determined on or after 60 days after the initiation of treatment and compared to a baseline prior to the initiation of drug administration. An assessment of the response to the agent can be made at any time following the first administration of the drug. For example, an assessment is made about 30 days, about 60 days, or about 90 after beginning treatment. Alternatively, assessment can be made about 6, 12, 18, 24 or more months after beginning treatment. Alternatively, an assessment can be

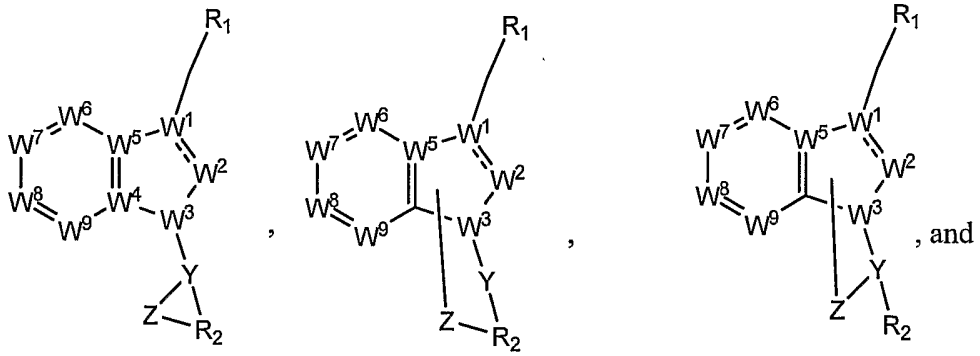
made less than about 30 days, about 30 days, about 60 days, or about 90 days after a course of treatment ends.

## 7. LONIDAMINE ANALOGS

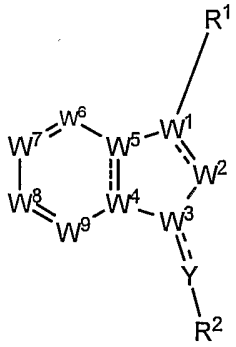
*Structure of lonidamine analogs*

**[0095]** Analogs of lonidamine can be used for prevention of cancer. Examples of lonidamine analogs include tolnidamine; AF-2364; and AF-2785 (see Ansari *et al.*, 1998; and Corsi *et al.*, 1976); and compounds described by Silvestrini, 1981; Lobl *et al.*, 1981; Cheng *et al.*, 2001; Andreani, F., 1984; Besner *et al.*, 1997; Caputo, A., 1981; Hagishita *et al.*, 1996; Kakehi *et al.*, 1978; Milanesio *et al.*, 2000; Palacios *et al.*, 1995; Silvestrini *et al.*, 1984; Tapia *et al.*, 1999; Wright and Collins, 1956: U.S. Patent Nos. 3,895,026; 6,001,865; 5,621,002; 4,002,749; 3,470,194; 3,625,971; 5,034,398; U.S. patent publication No. 2004/0167196; copending provisional patent applications 60/651705; 60/651671; 60/588,694, and the provisional Patent Application Matteucci *et al.*, entitled "Heterocyclic Lonidamine Analogs" Attorney Docket No. 021305-005900US filed 19 May 05 (incorporated by reference) and WO 96/03383. Synthesis of exemplary lonidamine analogs, including tolnidamine (TND), is described in the art including the aforelisted references.

**[0096]** In one embodiment, lonidamine analogs have the formula:



(I) (II) (III)



(IV)

**[0097]** wherein

**[0098]** $R^1$ is selected from the group consisting of $COOR^3$, $COR^4$, $CONR^3COR^3$, $CH=CHCO_2R^3$, $B(OR^3)_2$, $SO_2R^4$, $NHSO_2CR^5{}_3$, $NHSO_2CR^3{}_3$, $CONHSO_2CR^3{}_3$, $NHSO_2Ar$, $C(=NCN)NH_2$, $COCOR^4$ and $L^1$-$V^5$ wherein $L^1$ is selected from the group consisting of —C≡C—, $-C(V^1)=C(V^3)-$, $-C(V^1V^2)C(V^3V^4)-$, $-CV^1(CV^1V^2)_qCV^3-$, -NHCO- and -NHNH- wherein each $V^1$, $V^2$, $V^3$, and $V^4$ is independently selected from the group consisting of hydrogen, substituted or unsubstituted ($C_1$-$C_4$) alkyl or heteroalkyl, halogen, hydroxy, ($C_1$-$C_4$) alkoxy, cyano, nitro, amino, ($C_1$-$C_4$) alkylamino and ($C_1$-$C_4$) dialkylamino or $V^1$ and $V^3$ together form a cycloakyl, a heterocycloalkyl, a cycloalkenyl, an aryl or a heteroaryl ring; with the proviso that if one of $V^1$ and $V^2$ is hydroxyl, amino, ($C_1$-$C_4$) alkylamino or ($C_1$-$C_4$) dialkylamino, then the other is hydrogen or alkyl; and if one of $V^3$ and $V^4$ is hydroxyl, amino, ($C_1$-$C_4$) alkylamino, and ($C_1$-$C_4$) dialkylamino, then the other is hydrogen or alkyl; q is 1-6; $V^5$ is selected from $COOR^3$, $COR^4$, $CONR^3COR^3$, $COCOR^4$, $B(OR^3)_2$, $SO_2R^4$, $NHSO_2CR^5{}_3$, $NHSO_2CR^3{}_3$, $CONHSO_2CR^3{}_3$, $NHSO_2Ar$ and $C(=NCN)NH_2$; with the proviso that in $NHSO_2CR^5{}_3$, $R^5$ is not OH; when $L^1$ is -NHCO- then $V^5$ is $COR^4$, $NHSO_2CR^5{}_3$, $NHSO_2CR^3{}_3$, $NHSO_2Ar$ or $C(=NCN)NH_2$; and when $L^1$ is -NHNH- then $V^5$ is $COOR^3$, $COR^4$, $COCOR^4$, $B(OR^3)_2$, $SO_2R^4$, or $C(=NCN)NH_2$;

**[0099]** $R^2$ is an aryl or heteroaryl group, optionally substituted with from one to three $R^6$ substituents that are independently selected from the group consisting of halo and a straight or branched chain ($C_1$-$C_8$)alkyl;

**[0100]** $R^3$ is H, $(C_1\text{-}C_8)$alkyl or heteroalkyl, $(C_3\text{-}C_8)$cycloalkyl or heterocyclyl, or aryl or heteroaryl;

**[0101]** $R^4$ is $NR^3R^7$,$NR^3OR^7$, $NR^7NR^3R^7$ or $NR^3CN$;

**[0102]** $R^5$ is H, OH or halogen;

**[0103]** $R^7$ is H, $(C_1\text{-}C_8)$alkyl or heteroalkyl, $(C_3\text{-}C_8)$cycloalkyl or heterocyclyl, or aryl or heteroaryl;

**[0104]** $R^3$ and $R^7$ together are cycloalkyl, heteroalkyl or heteroaryl;

**[0105]** Ar is a substituted or unsubstituted aryl or heteroaryl;

**[0106]** each $W^1$, $W^3$, $W^4$ or $W^5$ is independently N or C;

**[0107]** $W^2$ is a member selected from the group consisting of N, $CR^5$, CO, O, $NR^7$ and S;

**[0108]** each $W^6$, $W^7$, $W^8$ or $W^9$ is independently N or $CV^6$ wherein $V^6$ is selected from the group consisting of hydrogen, substituted or unsubstituted $(C_1\text{-}C_4)$ alkyl or heteroalkyl, halogen, hydroxy, $(C_1\text{-}C_4)$ alkoxy, amino, cyano, nitro, $(C_1\text{-}C_4)_4$ alkylamino and $(C_1\text{-}C_4)$ dialkylamino;

**[0109]** Y is $CHR^8$, NH, $CR^8$, $NR^8$, S or O;

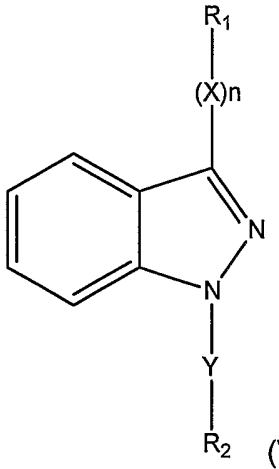
**[0110]** $R^8$ is H, a straight or branched chain $(C_1\text{-}C_8)$alkyl or heteroalkyl group;

**[0111]** ---------- represents a single, double or normalized bond; and

**[0112]** pharmaceutically acceptable salts, solvates, hydrates, and prodrugs thereof.

**[0113]** In one embodiment, lonidamine analogs have the formula

**[0114]**




(V)

**[0115]** where $R_1$, $R_2$, X, Y, and n are:

**[0116]** $R_2$ is -Cl, -Br, -I, or $-CH_3$, monosubstituted phenyl, substituted at the 2, 3, or 4 position; dichloro, dibromo, dimethyl, or chloro and methyl disubstituted phenyl, substituted at the 2 and 3 or 2 and 4 positions; or 2, 4, 5 trichlophenyl;

**[0117]** Y is $-(CH_2)-$; and

**[0118]** n is zero, and $R_1$ is -COOH, $-CONH_2$, $-CONHNH_2$, $-CONHN(CH_3)_2$, $-CH_2CH_2OH$, $-CH_2CH(OH)CH_2OH$, or $CH_2(CH_2OH)_2$; or

**[0119]** n is one, $R_1$ is -COOH, and X is -CH=CH-.

**[0120]** In one embodiment, the lonidamine analog used according to the present invention is other than tolnidamine and/or other than AF-2364 and/or other than AF-2785.

**[0121]**

**[0122]** *Activities of lonidamine analogs*

**[0123]** Functional characteristics of lonidamine analogs. Lonidamine analogs suited for use in the invention are those that interfere with cellular energy metabolism of prostate epithelial cells when administered to a human, non-human primate, or other mammal. As is usual in the pharmaceutical arts, not every structural analog of a compound (e.g., lonidamine) is pharmacologically active. Active forms can be identified by routine screening of analogs for the activity of the parent compound. A variety of assays and tests can be used to assess pharmacological activity of lonidamine analogs, including *in vitro* assays, such as those described below and elsewhere herein, *in vivo* assays of prostate function (including citrate production and ATP production) in humans, non-human primates and other mammals, *in vivo* assays of prostate size in humans, non-human primates and other mammals, and/or clinical studies.

**[0124]** Apoptosis assay in cell lines. As shown in Example 3, lonidamine induces apoptosis in cell lines derived from human prostate cells. The induction of apoptosis is significantly greater in LNCaP cells (ATCC NO. CLR-1740), a prostate-derived cell line that is citrate-producing, than in PC3 cells (ATCC NO. CLR-1435), a prostate-derived cell line that is citrate-oxidizing, consistent with the susceptibility of the citrate-producing prostate cells to metabolic inhibitors such as lonidamine. In some embodiments of the invention in which a lonidamine analog is used for treatment or prevention of BPH or its manifestations, an analog with similar apoptosis-inducing activity is selected. Thus, in some embodiments of the invention, a lonidamine

analog that induces apoptosis (enhances caspase 3 activity) in citrate-producing prostate cells, such as LNCaP cells, is administered to treat BPH. In some embodiments of the invention, a lonidamine analog that induces apoptosis in LNCaP cells to a significantly greater degree than in PC3 cells is administered to treat BPH. In some embodiments of the invention, the induction of apoptosis by the lonidamine analog is at least about 2-fold greater in LNCaP cells than in PC3 cells (and sometimes at least about 3-fold greater, at least about 4-fold greater, or at least about 10-fold greater) when assayed at the concentration of analog at which the difference in the level of apoptosis in the two cell lines is greatest (provided that the concentration of analog used in the assay is not greater than 1 mM).

**[0125]** Apoptosis assay in primary cell cultures. As shown in Example 2, lonidamine induces apoptosis in primary cultures of human prostate epithelial cells. The induction of apoptosis is significantly greater in primary cultures of prostate epithelial cells than in primary cultures of human prostate stromal cells, consistent with the susceptibility of citrate-producing prostate cells to metabolic inhibitors such as lonidamine. In some embodiments of the invention in which a lonidamine analog is administered for prevention of cancer, an analog with apoptosis-inducing activity similar to that of lonidamine is selected. Thus, in some embodiments of the invention, a lonidamine analog that induces apoptosis in prostate epithelial cells is administered to prevent cancer. In some embodiments of the invention, a lonidamine analog that induces apoptosis in primary cultures of prostate epithelial cells to a significantly greater degree than in primary cultures of human prostate stromal cells is used. In some embodiments of the invention, the lonidamine analog does not significantly induce apoptosis in stromal cells. In some embodiments of the invention, induction of apoptosis by the lonidamine analog is at least 2-fold greater in epithelial cells than in stromal cells (and sometimes at least 4-fold greater, sometimes at 10-fold greater, and sometimes at least 20-fold greater) when assayed at the concentration of analog at which the difference in the level of apoptosis in the two cell lines is greatest (provided that the concentration of analog used in the assay is not greater than 1 mM).

**[0126]** HIF-1alpha expression assays. As described in US 2004/0167196 and in Example 2, below, lonidamine reduced HIF-1 alpha expression/accumulation (measured in the nuclear fraction) in cells cultured under conditions of hypoxia by

almost 2-fold at 200 micromolar and by more than 5 fold (*i.e.*, more than 10-fold) at higher lonidamine concentrations. Thus, in some embodiments of the invention, an energolytic agent reduces HIF-1alpha expression (prevents HIF-1alpha accumulation) in LNCaP cells cultured under hypoxic conditions by at least about 2-fold, at least about 5-fold or at least about 10-fold compared to culture in the absence of lonidamine. The effect of lonidamine on HIF-1alpha expression in prostate cells appears more pronounced in LNCaP cells than in PC3 cells cultured under hypoxic conditions (oxygen level <0.1%). Some lonidamine analogs useful for prevention of cancer according to the present invention may have a similar effect.

**[0127]** Hexokinase activity. As discussed above, and without intending to be bound to any specific mechanism, the effects of lonidamine on the prostate may be mediated, at least in part, by its effects on mitochondria and mitochondrial hexokinase activity in secretory epithelial cells. Accordingly, some lonidamine analogs useful in the present invention have hexokinase inhibitory activity as great as or greater than that of lonidamine. Assays for hexokinase activity are known in the art. See Fanciulli *et al.*, 1996, and Floridi *et al.*, 1981.

**[0128]** Antispermatogenic activity. Likewise, it is believed that the anti-spermatogenic activity of lonidamine results, at least in part, from energolytic effects in germ cells. Some lonidamine analogs useful in the present invention have anti-spermatogenic activity as great, or greater, than that of lonidamine. Assays for anti-spermatogenic activity are known in the art. See, e.g., Grima *et al.*, 2001; Lohiya *et al.*, 1991.

**[0129]** In addition to *in vitro* assays, energolytic agents can be evaluated *in vivo* for use in the methods of the invention. For example and without limitation, suitable assays include measurements of prostate function and activity.

**[0130]** *In vivo* measurements of prostate function. The effect of a compound on prostate function, and, in particular, on respiration, can be assessed by monitoring prostate tissue metabolism following administration of the compound. Some lonidamine analogs useful in the present invention will detectably reduce ATP, citrate, and/or lactate production by the prostate in animals (including humans, non-human primates and other mammals). ATP, citrate, and/or lactate levels can be monitored directly and/or indirectly *in vivo* using techniques of magnetic resonance spectroscopy (MRS) or other methods. See, for example, Narayan and Kurhanewicz,

1992; Kurhanewicz *et al.*, 1991; Thomas *et al.*, 1990, for MRS assays that can be applied for this purpose. In one embodiment, the prostate function is assessed by measuring serum PSA levels. Some lonidamine analogs and other agents useful in the present invention will detectably reduce serum PSA levels when administered.

**[0131]** *In vivo* measurements of prostate size. The effect of a compound on prostate size can be assessed following administration of the compound using standard methods (for example, ultrasonography or digital rectal examination, for humans, and ultrasonography and/or comparison of organ weight in animals). Assays can be conducted in humans or, more usually, in healthy non-human animals or in monkey, dog, rat, or other animal models of BPH (see, Jeyaraj *et al.*, 2000; Lee *et al.*, 1998; Mariotti *et al.*, 1982), Some lonidamine analogs useful in the present invention will detectably reduce prostate size in such assays and animal models.

**[0132]** The activity of a lonidamine analog of interest in any of the aforementioned assays can be compared with that of lonidamine to provide guidance concerning dosage schedules for the compound, and other information. Generally, lonidamine analogs with greater biological activity per mg than lonidamine are of special interest.

**[0133]** In certain embodiments, the agent is a prodrug form of lonidamine, lonidamine analog, or other agent useful in the methods of the invention. Prodrug forms are known in the art and include of ester, amide and other derivatives of agents listed above. Exemplary prodrugs are described in copending US provisional application numbers 60/586,934 (filed July 8, 2004) and 60/624,505 (filed November 1, 2004). Also see US Pat. No. 6,146,658.

8. OTHER AGENTS THAT INTERFERE WITH CELLULAR ENERGY METABOLISM

**[0134]** A compound that inhibits or impairs energy production in prostate epithelial cells is referred to herein as an "energolytic agent." Such agents, in addition to lonidamine and lonidamine analogs, can be used for prevention of cancer. Energolytic agents useful in the practice of the invention include compounds that inhibit glycolysis, compounds that impair mitochondrial function, and compounds that do both, including, in all cases, compounds that act directly or indirectly on glucose metabolism in the prostate. Thus, in one embodiment, the energolytic agent is a compound that impairs glycolysis in prostate epithelial cells. In one embodiment, the energolytic agent is a compound that impairs mitochondrial function in prostate

epithelial cells. In one embodiment, the energolytic agent interferes with both glycolysis and mitochondrial function. In one embodiment, a combination of agents is used, including, in one embodiment, the administration of one agent that is an inhibitor of glycolysis and simultaneous or contemporaneous administration of a second agent that is an inhibitor of mitochondrial function. One class of energolytic agents includes compounds that inhibit glycolysis (directly or indirectly).

**[0135]** For example, the agent may inhibit an enzyme that catalyses a step in the conversion of glucose to pyruvate, or the oxidation of pyruvate to acetyl-CoA. For example, and not for limitation, the agent may be an inhibitor of hexokinase, glucokinase, phosphofructokinase, aldose, phosphoglycerate kinase, enolase, pyruvate kinase and/or pyruvate dehydrogenase. For illustration and not limitation such compounds include those described in U.S. Patent No. 5,824,665 (e.g., 6-amino-6-deoxy-glucose; N-acetyl-β-D-mannosamine; D-mannosamine; N-α-(p-tosyl)-L-lysine chloromethyl ketone); phosphoglycerate; quinone methides; taxodone; taxodione; α-methylene lactones; euparotin acetate; eupacunin; vernolepin; argaric acid; quinaldic acid; 5'-p-fluorosuflonylbenzoyl adenosine; 5-keto-D-fructose; 5-keto-D-fructose-1,6-bisphosphate; Mg-phosphoglycerate; 2,3-diphosphoglycerate; 3-(trans)-chlorophosphoenolpyruvate; 3-(cis)-cyanophosphoenolpyruvate; D-tartronate; semialdehyde phosphate; aminoenolpyruvate; D-glycidol phosphate; L-glycidol phosphate; hydroxy-1-cyclopropanecarboxylic acid; D(-)3-phosphoglyceric acid; glyoxylate; hydroxypyruvate; kynurenate; xanthurenate; α-cyano-4-hydroxycinnamic acid; bromopyruvic acid; fluropyruvic acid) or pharmaceutically acceptable analogs or derivatives thereof. *See,* Bisswanger, 1981; Furuta and Hashimoto, 1982; Waymack *et al.*, 1979; Lowe and Perham, 1984; Bisswanger, 1980; Colombo, 1975; Hanson *et al.*, 1970; McCune *et al.*, 1989; Mansour and Colman, 1978; Avigad and Englard, 1974; Scopes and Stoter, 1982; Gunter, 1982; Liu *et al.*, 1990; Wirsching and O'Leary, 1985; Spring and Wold, 1971; Rose and O'Connell, 1969; O'Leary *et al.*, 1981; de Domenech, 1980; and Johnson *et al.*, 1982.

**[0136]** Another class of agents includes compounds that impair mitochondrial function (e.g., a mitochondrial poison). Mitochondrial poisons include but are not limited to the mitochondrial poisons described in U.S. Patent No. 6,670,330.

**[0137]** As noted above, some agents may interfere with both glycolysis and mitochondrial function. It will also be appreciated that agents that impair glycolysis

will generally also at least indirectly reduce energy production by mitochondria, by reducing the amount of pyruvate available for entry into the TCA cycle.
Several exemplary agents are discussed below.

A) 2-Deoxyglucose and Analogs of 2-Deoxyglucose

**[0138]** One agent suitable for use in the methods of the present invention is 2-deoxy-D-glucose (2-DG). 2-DG is phosphorylated by hexokinase to produce 2-DG-6-phosphate, which is not further metabolized and which inhibits hexokinase. 2-DG has been shown to inhibit glycolysis in cancer cells.

**[0139]** Another example of an agent is an analog of 2-DG that has glycolysis inhibiting activity. As used herein, a 2-DG analog is any D-glucose analog other than 2-DG that does not have a hydroxyl group at the 2 position of the glucose ring. L-glucose and its L-analogs are not 2-DG analogs for purposes of the present invention. A glucose analog includes mannose, galactose, glucose, and 5-thio-glucose. An analog of glucose or 2-DG can have a fluorine in place of a hydrogen at any position on the glucose ring; thus, 2-fluoro-2-deoxy-D-glucose (2-FDG) and 2-difluoro-2-deoxy-D-glucose are 2-DG analogs. An analog of glucose or 2-DG can have an amino group in place of a hydroxyl group at any position on the glucose ring other than the 6 position; thus, 2-amino-2-deoxy-D-glucose (2-glucosamine) and 2-amino-2-deoxy-D-galactose (2-galactosamine) are 2-DG analogs. Other illustrative 2-DG analogs include 2-F-mannose, 2-mannosamine, 2-deoxygalactose, 2-F-deoxygalactose, and di, tri, and other oligosaccharades that contain one or more of the preceding or following 2-DG analogs. Other 2-DG analogs useful in the methods of the present invention include the analogs described in U.S. patent application Serial No. 10/754,239, now U.S. patent publication No. US 2004/0167079, both incorporated herein by reference. 2-DG analogs useful in the present invention also include those analogs described in Reinhold, 2000, *Oncol. Rep.,* 7:1093-97 (e.g., 2-deoxy-D-glucose tetraacetate) and in PCT publication WO 01/82926.

*Dosage Schedules for 2-DG and Analogs*

**[0140]** In the case of 2-deoxyglucose (2-DG) and analogs (2-DGA) thereof, exemplary dosage schedules are described in copending U.S. patent application No. 10/754,239, filed January 9, 2004, now U.S. patent publication No. US 2004-

0167079 A1, incorporated herein by reference. For example, 2-DG and 2-DGA can be administered for the prevention of prostate cancer at a dose in the range of about 1 mg to about 2 g of 2-DG or 2-DGA per kg of body weight of the patient to be treated. In another embodiment, 2-DG or a 2-DGA is administered in a dose in the range of about 10 mg to about 1 g of 2-DG or a 2-DGA per kg of body weight of the patient to be treated. In certain other embodiments, 2-DG or a 2-DGA is administered in a dose of about 50 to 250 mg of a 2-DG or a 2-DGA per kg of body weight of the patient to be treated. In another embodiment, a prophylactically effective dose is about 25 mg/kg to about 150 mg/kg. For illustration, the prophylactically effective dose of 2-DG or a 2-DGA is administered daily or once every other day or once a week to the patient, and multiple administrations of the drug are employed. Depending on the dose selected by the practitioner and the convenience of the patient, the entire dose may be administered once daily, or the dose may be administered in multiple smaller doses through the course of a day. For example, the dose may be divided into two smaller doses and administered twice daily, or divided into three smaller doses and administered thrice daily. Alternatively, the dose may be combined and given every other day, or even less frequently, but in any event, the dose is repeatedly administered over a period of time. For optimum treatment benefit, the administration of the prophylactically effective dose is continued for multiple days, typically for at least five consecutive days, and often for at least a week and often for several weeks or more. In one embodiment, 2-DG or a 2-DGA is administered once (qday), twice (bid), three times (tid), or four times (qid) a day or once every other day (qod) or once a week (qweek), and treatment is continued for a period ranging from three days to two weeks or longer. In one embodiment, the treatment is continued for one to three months. In another embodiment, the treatment is continued for a year. Thus, a patient may be administered 2-DG or a 2-DGA for a week, a month, two months, three months, six months, or a year or longer.

B) 3-Bromopyruvate and its analogs

**[0141]** Another class of agents suitable for use in the methods of the present invention is the class of 3-halo-pyruvates, including but not limited to 3-bromopyruvate, an inhibitor of hexokinase. For additional information on 3-halo-

pyruvates, see U.S. Patent No. 6,670,330 and U.S. patent publication No. 2003/0087961.

C) Gossypol and gossypol analogs

**[0142]** Another class of agents suitable for use in the methods of the present invention is the class composed of gossypol and its analogs, including but not limited to gossypol-(+), gossypol-(-), mixtures of gossypol-(+) and –(-), gossypol acetic acid, gossypol aldehyde, gossypol hemiacetal, gossypol quinoid, gossypolone, metabolites thereof, and physiologically acceptable salts thereof. Gossypol, gossypol analogs, formulations, and unit dose forms that can be employed in the methods of the present invention are described in PCT patent publication Nos. WO 02/097053; WO 02/47673; U.S. Patent Nos. 6,114,397 and 4,381,298; and U.S. patent publication No. 2002/137801.

D) Other agents

**[0143]** Other useful glycolytic inhibitors, mitochondrial function inhibitors, mitochondrial poisons, and hexokinase inhibitors useful in the methods of the present invention are known or can be identified using assays known in the art or described herein. For example, compounds for use as agents in the present invention include those described in PCT patent publication WO 01/82926 and U.S. Patent Nos. 6,670,330; 6,218,435; 5,824,665; 5,652,273; and 5,643,883; and U.S. patent application publication Nos. 2003/0072814; 2002/0077300 (e.g., apoptolidin); and 2002/0035071.

**[0144]** Additional agents for use as agents in the present invention include the anti-metabolites described in U.S. patent publication No. 2002/0035071 (Pitha) including 3-O-methylglucose (Jay *et al.*, 1990); anhydrosugars such as 1,5-Anhydro-D-Glucitol (Polygalitrol) (Sols *et al.*, 1954), 1,5-anhydroglucitol-6-phosphate (Crane *et al.*, 1954) and, 2.5-Anhydro-D-Mannitol and 2,5-Anhydroglucitol).

**[0145]** Compounds for use as agents in the present invention also include inhibitors of lactate dehydrogenase, such as oxamate, and inhibitors of glyceraldehyde 3-phosphate dehydrogenase, such as iodoacetate (see, Lampidis, WO 01/82926).

**[0146]** Any of a variety of agents may be used for prevention of prostate cancer. In one embodiment, the agent is an inhibitor of hexokinase. In one embodiment, the agent is an inhibitor of glucokinase. In one embodiment, the agent is an inhibitor of phosphofructokinase. In one embodiment, the agent is an inhibitor of aldose. In one embodiment, the agent is an inhibitor of phosphoglycerate kinase. In one embodiment, the agent is an inhibitor of enolase. In one embodiment, the agent is an inhibitor of pyruvate kinase. In one embodiment, the agent is an inhibitor of pyruvate dehydrogenase. In one embodiment, the agent is an inhibitor of lactate dehydrogenase. In one embodiment, the agent is an inhibitor of glyceraldehyde 3-phosphate dehydrogenase. In one embodiment, the agent is an inhibitor of glucose transport. In one embodiment, the agent reduces glucose transporter levels or prevents those levels from rising. In one embodiment, the agent is 2-deoxy-D-glucose (2-deoxyglucose or 2-DG). In one embodiment, the agent is an analog of 2-deoxyglucose. In one embodiment, the agent is 2-deoxy-D-glucose tetraacetate or 5-thio-glucose. In one embodiment, the agent is gossypol. In one embodiment, the agent is a gossypol analog. In one embodiment, the agent is 3-bromopyruvate. In one embodiment, the agent is a 3-bromopyruvate analog. In one embodiment, the agent is an analog of lonidamine. In one embodiment, the agent is lonidamine. In one embodiment, the agent is tolnidamine. In one embodiment, the agent is oxamate. In one embodiment, the agent is iodoacetate. In one embodiment, the agent is apoptolidin. In one embodiment, the agent is an analog of apoptolidin.

**[0147]** In one embodiment, the agent has a molecular weight less than 1000, optionally less than 500. In one embodiment, the agent is synthetic and does not occur in nature.

**[0148]** In one embodiment, the agent is other than an inhibitor of hexokinase. In one embodiment, the agent is other than an inhibitor of glucokinase. In one embodiment, the agent is other than an inhibitor of phosphofructokinase. In one embodiment, the agent is other than an inhibitor of aldose. In one embodiment, the agent is other than an inhibitor of phosphoglycerate kinase. In one embodiment, the agent is other than an inhibitor of enolase. In one embodiment, the agent is other than an inhibitor of pyruvate kinase. In one embodiment, the agent is other than an inhibitor of pyruvate dehydrogenase. In one embodiment, the agent is other than an inhibitor of lactate dehydrogenase. In one embodiment, the agent is other than an

inhibitor of glyceraldehyde 3-phosphate dehydrogenase. In one embodiment, the agent is other than an inhibitor of glucose transport. In one embodiment, the agent does not reduce glucose transporter levels or prevent those levels from rising. In one embodiment, the agent is not a direct or indirect inhibitor of HIF-1alpha.

**[0149]** In one embodiment, the agent is other than 2-deoxyglucose. In one embodiment, the agent is other than an analog of 2-deoxyglucose. In one embodiment, the agent is other than 2-deoxy-D-glucose tetraacetate. In one embodiment, the agent is other than 5-thio-glucose. In one embodiment, the agent is other than gossypol. In one embodiment, the agent is other than a gossypol analog. In one embodiment, the agent is other than 3-bromopyruvate. In one embodiment, the agent is other than a 3-bromopyruvate analog. In one embodiment, the agent is other than lonidamine. In one embodiment, the agent is other than tolnidamine. In one embodiment, the agent is other than an analog of lonidamine. In one embodiment, the agent is other than an inhibitor of aconitase.

**[0150]** In one embodiment, the agent is other than oxamate. In one embodiment, the agent is other than iodoacetate. In one embodiment, the agent is other than apoptolidin. In one embodiment, the agent is other than analog of apoptolidin.

**[0151]** In some embodiments the agent is other than zinc or any other than any agent known for use for prevention of prostate cancer on June 30, 2004, without regard to whether or not the agent is known to inhibit glycolysis or impair mitochondrial function.

**[0152]** Although for illustration a wide variety of agents have been described herein, it will be appreciated that an agent suitable for use according to the invention for prevention of prostate cancer will be pharmaceutically acceptable, *i.e.*, will not be toxic to the subject at the doses and formulation administered, or the detrimental effects of any toxicity (e.g., side effects) associated with the agent will be outweighed by the benefit to the subject. Methods for identification and assessment of pharmaceutically acceptable agents are well known in the medical and pharmaceutical arts. For example, the therapeutic (i.e., prophylactic) index (i.e., dose ratio of therapeutic effects to toxic effects, which can be expressed as the $ED_{50}$ /$LD_{50}$ ratio) can be estimated using cell culture assays and animal studies. The data obtained are used to formulate a range of dosage for human use. Pharmaceutical compositions which exhibit large therapeutic indices are preferred.

**[0153]** In certain embodiments, the agent is a pharmaceutically acceptable salt of an agent named above. Pharmaceutically acceptable salts include addition salts with acids, as well as the salts with bases. Salts with bases are, for example, alkali metal or alkaline earth metal salts, such as sodium, potassium, calcium or magnesium salts, or ammonium salts, such as those with ammonia or suitable organic amines, e.g. diethylamine, di-(2-hydroxyethyl)-amine or tri-(2-hydroxyethyl)-amine. Suitable acids for the formation of acid addition salts are, for example, mineral acids, such as hydrochloric, hydrobromic, sulphuric or phosphoric acid, or organic acids, such as organic sulphonic acids, for example, benzenesulphonic, 4-toluenesulphonic or methanesulphonic acid, and organic carboxylic acids, such as acetic, lactic, palmitic, stearic, malic, maleic, fumaric, tartaric, ascorbic or citric acid.

9. ASSAYS

**[0154]** In one aspect, the invention provides methods for determining the usefulness of a compound for prevention of prostate cancer. In one embodiment, the method involves (a) contacting a citrate-producing cell with the compound; (b) contacting a citrate-oxidizing cell with the compound; and (c) detecting a differential effect of said contacting on the citrate-producing cell compared to the citrate-oxidizing cell. A differential effect (e.g., as described herein) indicates that the agent may be useful for prevention of prostate cancer. Further and confirmatory assays can then be conducted. The method can be conveniently carried out *in vitro*.

**[0155]** These methods were developed, in part, based on the discovery that the energolytic agent lonidamine induces apoptosis of prostate cells, and that the induction is substantially more pronounced in citrate-producing cells compared to citrate-oxidizing cells and that lonidamine reduces expression (accumulation) of HIF-1 alpha in prostate cells, especially under hypoxic conditions, and that induction of apoptosis in prostate epithelial cells can reduce the likelihood or prevent prostate cancer.

**[0156]** A variety of cells and assays can be used in the methods of the invention. Citrate-producing and citrate-oxidizing cell types are known and can be identified using art-known assays and criteria. See, e.g., Costello and Franklin, 1997, *Urology 50*:3-12 and Franklin *et al.*, 1995, *Endocrine 3*:603-607. Suitable citrate-producing cells include primary cultures of prostate epithelial cells and certain established cell

lines derived from prostate epithelial cells (e.g., LNCaP cells). Suitable citrate-oxidizing cells include primary cultures of prostate stromal cells and certain established cell lines derived from prostate cells (many malignant prostate epithelial cells undergo an apparent metabolic transformation from citrate-producing to citrate-oxidizing; see Franklin *et al.*; 1995, *Endocrine* 3:603-607). In one embodiment, the citrate-producing cell is an LNCaP cell and the citrate oxidizing cell is a PC-3 cell. Primary cultures of human prostate epithelial and stromal cells are commercially available (e.g., cells can be obtained from Cambrex Bio Science Rockland, Inc., 191 Thomaston Street, Rockland, Maine 04841) and can be prepared according to well known tissue culture methods (see, e.g., Peehl, DM, Culture of Epithelial Cells: Prostate Culture, 1992, 159-180). Established cell lines derived from prostate are available from the American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, VA 20108 USA, or can be prepared according to well known methods. In another embodiment, the cell is a recombinant cell selected from the group consisting of (i) a cell, optionally a cell other than a prostate cell, that has been modified so as to accumulate zinc to levels that inhibit m-aconitase and (ii) a cell other than a prostate cell that cannot metabolize citrate. In one embodiment, the cell is a cell that has been modified to not express m-aconitase or to express only an inactive mutant thereof. In one embodiment, the cells are human (although cells from other mammals also can be used). In one embodiment, the cells have been immortalized by modification such that telomerase expression occurs constitutively.

**[0157]** In a typical assay, cells are contacted with the test compound, usually at a range of concentrations (e.g., 10, 50, 100, 200, 400, 600 and 800 microM). The contacting is conveniently achieved by adding the compound to the medium in which the cells are cultured, or any other method of contacting. In one embodiment, the compound is introduced into the cell or cell culture in a carrier (e.g., liposomal carrier) or solvent. It will be understood that, as is usual in drug screening assays, suitable controls (e.g., negative controls) and statistical methods are used. Assays can be carried out on whole cells, cell extracts or, alternatively, nuclear extracts.

**[0158]** It has been discovered that the some of the differential effects of lonidamine on citrate-producing and citrate-oxidizing cells are most striking in cells grown under conditions of hypoxia. Accordingly, in some embodiments of the invention, the cells are grown under hypoxic conditions. For example, cells can be

cultured in low oxygen levels (e.g., <0.1%). Hypoxia can also be induced by culture at high cell density.

**[0159]** An example of a differential effect is induction of apoptosis that is greater in citrate-producing cells compared to citrate-oxidizing cells. In one embodiment the differential effect is induction of apoptosis that is greater in citrate-producing cells compared to citrate-oxidizing cells. In one embodiment, the differential effect is at least about 10-fold or at least about 20-fold. A number of assays for apoptosis or its markers or other indicators thereof are known and can be used in the present assay. For example and not limitation, apoptosis assays include assays for caspase 3, DNA fragmentation assays (e.g., TUNEL assays; BD Biosciences No 556381), and Annexin V assays (e.g. BD Biosciences No 556547).

**[0160]** The effect of lonidamine on HIF-1alpha expression in prostate cells appears more pronounced in LNCaP cells than in PC3 cells when cultured under hypoxic conditions (oxygen level <0.1%). Some energolytic agents useful for treatment of BPH according to the present invention may have a similar effect. Accordingly, another differential effect that can be measured is a reduction in HIF-1 alpha expression that is greater in citrate-producing cells than in citrate-oxidizing cells, especially cells cultured under hypoxic conditions. For example, the difference is at least about 2-fold, and sometimes at least about 4-fold.

## 10. EXAMPLES

### Example 1: Administration of Lonidamine to Men with Benign Prostate Hyperplasia Reduces Prostate Volume

**[0161]** This example describes results of a Phase II Open-Label Study to evaluate the efficacy and safety of oral lonidamine (LND) treatment in subjects with symptomatic benign prostatic hyperplasia (BPH) by measuring the change from baseline of: total prostate volume by transrectal ultrasound (TRUS), maximum flow rate ($Q_{max}$) on uroflowmetry, international prostatic symptoms score (I-PSS) and prostate-specific antigen (PSA). Thirty subjects with symptomatic BPH received oral LND (150 mg/day) once daily for 28 days. Subjects were assessed at baseline, at active-therapy assessment visits (Day-14, Day-28), and four weeks post-therapy (Day-56) for prostate volume by TRUS, $Q_{max}$, I-PSS, PSA, serum chemistry and adverse events. Subjects experienced an average decrease of 11.2% ($p<0.001$) in

prostate volume on Day-28, which was maintained at Day-56 (average decrease of 12.0%; $p<0.001$). $Q_{max}$ improved by a mean of 3.2 mL/sec at Day-28 ($p=0.002$). I-PSS scores improved from 19.5 prior to treatment to 12.2 at Day-28 ($p<0.001$). PSA decreased on average by 17.8% from baseline ($p=0.001$) to Day-28. LND was generally well-tolerated, with no treatment-related moderate or severe adverse events.

*Study Design*

**[0162]** 30 male subjects male subjects between 50 and 80 years of age were enrolled and received LND therapy. Subjects were eligible for inclusion if they had experienced lower urinary tract symptoms (LUTS) for at least 3 months, had a prostate volume >30 cc as measured by TRUS, a $Q_{max}$ < 15 mL/sec as measured by uroflowmetry, an I-PSS of >13, PSA > 1.0 ng/mL, and were able to comply with the prescribed treatment protocol and evaluations. Exclusion criteria included prior therapy for BPH other than alpha-blockers; prior surgery of the prostate (except biopsies); uncontrolled diabetes mellitus (fasting blood glucose > 200 mg/dL); current or past evidence of malignant disease of the prostate; active cardiac, renal, or hepatic disease as evidenced by creatinine ≥1.8 mg/dL, ALT or AST ≥2.5x the upper limit of normal, history of myocardial infarction, congestive heart failure, or unstable cardiac arrhythmias within 6 months prior to study entry. Alpha-blocker therapy was not allowed during or for 14 days prior to the study. All subjects gave informed consent according to institutional guidelines.

**[0163]** Pretreatment evaluation included recording of medications taken 2 weeks prior to study entry; serum chemistry panel including sodium, potassium, chloride, carbon dioxide, blood urea nitrogen (BUN), creatinine, AST and ALT; hormonal profile (FSH, LH, PRL, testosterone, free testosterone); TRUS measurement of prostate volume; PSA; uroflowmetry; and I-PSS. On Day-14 and Day-28 subjects were evaluated for prostate volume, PSA, uroflowmetry, adverse event and concomitant medication assessments. On Day-28, subjects also underwent a physical examination including digital rectal exam, temperature and vital signs; evaluation of serum chemistry; hormonal profile; and I-PSS. On Day-56, subjects were also evaluated for prostate volume and uroflowmetry. On Day-84 and Day-112, subjects were evaluated for uroflowmetry.

**[0164]** The primary efficacy endpoints were the change from baseline to Day-28 in prostate volume, $Q_{max}$, I-PSS and PSA. Efficacy was analyzed by paired t-tests of the percent change in prostate volume and PSA and absolute change in $Q_{max}$ and I-PSS from baseline. Non-parametric tests were also performed to examine the robustness of the results. All tests were two-sided and no adjustments were made for multiple tests. Missing data for Day-14 and Day-28 were replaced using the last observation carried forward procedure.

*Prostate volume*

**[0165]** Mean total prostate volume decreased significantly during the course of treatment (Figure 1; Table 2). At the end of the treatment (Day-28) 16 subjects (53%) had ≥10% and 7 subjects (23%) had ≥20% reduction in total prostate volume, respectively. Prostate volume continued to decrease even after subjects had been off LND therapy for four weeks.

*PSA*

**[0166]** At Day-14, PSA levels decreased modestly on average by 1.5% (95% CI: -14.1% to 11.2%), but by Day-28 PSA levels had dropped on average 17.8% (95% CI: -27.2% to –8.4%; $p<0.001$). (Figure 2; Table 2). Without intending to be bound by a particular mechanism, it is believed that the increased PSA resulted from destruction of lonidamine sensitive cells in the prostate.

*Uroflowmetry, I-PSS, $Q_{max}$*

**[0167]** Subjects experienced a significant increase in $Q_{max}$ by Day-14 that continued through Day-28 and Day-56. At the end of the treatment 12 subjects (40%) had more than 3 mL/sec and 7 subjects (23%) more than 5 mL/sec improvement over baseline $Q_{max}$. The residual urine (post-void volume) decreased significantly from an average volume of 82.1 mL at baseline to 44.0 mL at Day-14, 31.6 mL at Day-28, and 34.8 mL at Day-56.

**[0168]** Subjects' I-PSS symptom scores decreased from an average of 19.5 at baseline to 12.2 on Day-28 ($p <0.001$). The decreases were consistent across subjects.

**[0169]** Prostate volume and BPH-related symptoms continued to improve throughout the 28 days of LND treatment and into the post-treatment period, with further reduction of prostate volume and improvement of $Q_{max}$ at Day-56. Baseline equals Day 0 value.

TABLE 2: Summary of Efficacy Endpoints

| Prostate Volume | | | | | |
|---|---|---|---|---|---|
| Day | N | Mean (cc) | SD (cc) | Mean Absolute Change from Baseline (cc) | Mean Percent Change from Baseline |
| 0 | 30 | 55.4 | 25.4 | NA | NA |
| 14 | 30 | 52.0 | 25.0 | -3.4 | -6.5% |
| 28 | 29[b] | 49.6 | 24.5 | -5.9 | -11.2% |
| 56 | 28 | 48.2 | 23.5 | -6.2 | -12.0% |
| PSA | | | | | |
| Day | N | Mean (ng/mL) | SD (ng/mL) | Mean Absolute Change from Baseline (ng/mL) | Mean Percent Change from Baseline |
| 0 | 30 | 3.6 | 1.9 | NA | NA |
| 14 | 28[b] | 3.6 | 2.2 | 0.0 | -1.5% |
| 28 | 29[b] | 2.8 | 1.5 | -0.7 | -17.8% |
| I-PSS (score in units) | | | | | |
| Day | N | Mean (units) | SD (units) | Mean Absolute Change from Baseline (units) | Mean Percent Change from Baseline |
| 0 | 30 | 19.5 | 2.9 | NA | NA |
| 28 | 27[a] | 12.2 | 4.4 | -7.3 | -37.6% |
| Flow Rate (mL/sec) | | | | | |
| Day | N | Mean (mL/sec) | SD (mL/sec) | Mean Absolute Change from Baseline (mL/sec) | Mean Percent Change from Baseline |
| 0 | 30 | 9.4 | 2.7 | NA | NA |
| 14 | 28[b] | 12.5 | 5.5 | 3.1 | 38.2% |
| 28 | 29[b] | 12.6 | 6.0 | 3.2 | 35.3% |
| 56 | 27 | 12.7 | 5.4 | 3.3 | 38.3% |
| 84 | 27 | 12.9 | 5.5 | 3.4 | 37.3% |
| 112 | 27 | 13.7 | 5.9 | 4.2 | 48.2% |
| Post Micturitional Residue (mL) | | | | | |
| Day | N | Mean (mL) | SD (mL) | Mean Absolute Change from Baseline (mL) | Mean Percent Change from Baseline |
| 0 | 30 | 82.1 | 64.1 | NA | NA |
| 14 | 28[b] | 44.0 | 62.5 | -38.1 | -45.7% |
| 28 | 29[b] | 31.6 | 45.3 | -50.5 | -46.6% |
| 56 | 28 | 34.8 | 41.1 | -44.9 | -49.6% |
| 84 | 27 | 37.6 | 52.7 | -42.1 | -44.9% |
| 112 | 27 | 47.3 | 43.7 | -32.5 | -30.8% |

[a] Last observation carried forward for 1 patient without Day 28 data and 2 patients with partially incomplete Day 28 data (analyses include 30 patients).
[b] Last observation carried forward for patients with missing data (analyses include 30 patients).

Example 2: Lonidamine Induces Apoptosis in Citrate-Producing Cells

**[0170]** To determine whether apoptosis occurs in cells treated with lonidamine, the effect of lonidamine on cells producing citrate (LNCaP) and cells oxidizing citrate (PC3) was assessed. Lonidamine induced-apoptosis (as measured by activation of caspase 3) in citrate-producing cells (LNCaP) to a much greater extent than in citrate-oxidizing cells (PC3). The activation of caspase3 was observed to be a time-dependent process, as measured by lonidamine-induced apoptosis of citrate-producing cells.

The effect of lonidamine was also examined in primary cultures of prostate epithelial cells (which accumulate citrate) or prostate stromal cells (which do not accumulate citrate). Lonidamine induced apoptosis only in prostate epithelial cells in a dose-dependent manner. In contrast, induction of apoptosis was not observed in prostate stromal cells after treatment with lonidamine.

Methods:

**[0171]** Immunoblotting: To detect the expression of caspase 3, the membrane was blocked with TBST containing 5% non-fat milk for 1 h at room temperature, and caspase 3 protein was detected by incubation with caspase 3 antibody overnight at 4°C and with the alkaline phosphatase-conjugated secondary antibody for 1 h. The specific protein was detected using colorimetric substrate, and the intensity of each protein was quantified using an NIH image system.

**[0172]** Primary Cell Cultures: Primary cultures of human prostate epithelial cells (Cambrex No. CC-2555) and human prostate stromal cells (Cambrex No. CC-2508) were obtained from Cambrex Bio Science Rockland, Inc. (191 Thomaston Street, Rockland, Maine 04841).

**[0173]** Apoptosis Assay: Cells were plated at a density of $2 \times 10^4$ cells per well in a 96 well plate, and then maintained in a 37°C incubator (5% $CO_2$) for 16 h. Lonidamine was added into each well at different concentrations, and then incubated for 6 h at 37°C. To assess the caspase 3 activity, the homogeneous buffer and

caspase 3 substrate (Promega No G7791; Promega Corporation, 2800 Woods Hollow Road, Madison WI USA 53711) were added into each well in the presence or absence of caspase 3 inhibitor (Promega No G5961). The fluorescence intensity of cleaved substrate was determined using a fluorescence plate reader at excitation 485 nm and emission 530 nm.

Example 3: Administration of Lonidamine Reduces the Incidence of Mammary Tumors in Sprague Dawley Rats

**[0174]** A GLP study was conducted to evaluate the effect of lonidamine on the incidence of tumors in Sprague Dawley rats. A total of 500 rats were assigned to groups of 50 rats of each sex per dosage level. Two groups of each sex served as controls and the other six groups (three of each sex) received lonidamine (20, 60 or 180 mg/kg per day) in the diet for 2 years. At the end of the two year study period, the rats were sacrificed and tissues were obtained at necropsy for histological assessment including determination of tumor incidence. Survival probability functions were estimated by the Kaplan-Meier technique.

**[0175]** In the control groups, the incidence of tumor-bearing animals was 90% in the males and 92% in the females. In the high lonidamine dose group animals, there was no increase in the incidence of tumor-bearing animals (Table 3). In fact, in high dose group females there was a slight reduction in the numbers of both benign and malignant tumors. In the high dose group the incidence of mammary tumors was statistically decreased (P = 0.0004) in the females (Table 4). The results of this study indicate that lonidamine administration can reduce the likelihood of developing certain types of epithelial cancer.

Table 3: Number of Rats with Tumors

| Lonidamine Dose (mg/kg) | Primary Neoplasms | | Benign Neoplasms | | Malignant Neoplasms | |
|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female |
| Control I | 46 | 46 | 45 | 43 | 7 | 8 |
| 20 | 42 | 45 | 37 | 44 | 11 | 7 |
| 60 | 40 | 38 | 38 | 36 | 6 | 8 |
| 180 | 48 | 40 | 42 | 30 | 8 | 4 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Control II | 44 | 46 | 38 | 44 | 11 | 6 |

Table 4: Incidence of Mammary Tumors in Female Rats

| Lonidamine Dose (mg/kg) | # of rats with mammary tumors/total # of rats examined |
|---|---|
| Control I | 22/48 |
| 20 | 14/32 |
| 60 | 18/29 |
| 180 | 7/49* |
| Control II | 27/50 |

*p < 0.001 vs. Control

11. REFERENCES CITED

**[0176]** Each of the publications and patent documents cited herein are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference.

**[0177]**

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**[0303]** US 2002/151470 "Inhibitors of phospholipase A2"

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**[0305]** US 2002/0077300 "Screening Method for Cancer Therapeutics and Stable Antitumor Drug"

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**[0307]** US 6,902,892 "Method of diagnosing, monitoring, staging, imaging and treating prostate cancer"

**[0308]** US 6,670,330 "Cancer Chemotherapy With 2-Deoxy-D-Glucose"

**[0309]** US 6,146,658 "Prodrugs, their preparation and use as pharmaceuticals"

**[0310]** US 6,114,397 "Gossypol for the Treatment of Cancer"

**[0311]** US 6,001,865 "3-Substituted 1-Benzyl-1H-Indazole Derivatives as Antifertility Agents"

**[0312]** US 5,824,665 "Reduction of Hair Growth".

**[0313]** US 5,665,874 "Cancer related antigen "

**[0314]** US 5,652,273 "Reduction of Hair Growth".

**[0315]** US 5,643,883 "Glucose-6-Phosphate Uptake Inhibitors and Novel Uses Thereof".

**[0316]** US 5,621,002 "Prodrugs for Enzyme Mediated Activation"

**[0317]** US 5,260,327 "Methods for inhibiting the proliferation of brain and hepatic metastases by using lonidamine"

**[0318]** US 5,034,398 "1H-Indazole-3-Carboxamide-N-2-Azabicyclo[2.2.2]Octanes Useful for Treating Anxiety, Psychosis, Neuralgia, Migraine and Cluster Headaches"

**[0319]** US 4,381,298 "Inductive Torque Transmitter With Stationary Field Winding"

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**[0321]** US 3,895,026 "Substituted 1-benzyl-1H-indazole-3-carboxylic Acids and derivatives thereof"

**[0322]** US 3,625,971 "Lower Alkylesters of 1-(Disubstituted Phenyl or Benzyl)-1I-Indazol-3-Yloxy Acetic Acids"

**[0323]** US 3,470,194 "Indazole-3-YL)-Oxyalkanoic Acids"

**[0324]** WO 04/064735 "Treatment of Benign Prostatic Hyperplasia"

**[0325]** WO 04/064736 "Treatment of Benign Prostatic Hyperplasia Using Energolytic Agents"

**[0326]** WO 03/064593 "Antigen Panels and Method of Using the Same"

**[0327]** WO 0247673 "Methods and Compositions to Treat Conditions Associated With Neovascularization"

**[0328]** WO 02/097053 "Small Molecule Antagonists of BCL2 Family Proteins"

**[0329]** WO 01/82926 "Manipulation of Oxidative Phosphorylation for Hypersensitizing Tumor Cells to Glycolytic Inhibitors"

**[0330]** WO 98/037093 "Compounds for Immunotherapy of Prostate Cancer and Methods for Their Use"

**[0331]** WO 98/45436 "Secreted Expressed Sequence Tags (sESTs)"

**[0332]** WO 98/45435 "Secreted Expressed Sequence Tags (sESTs)"

**[0333]** WO 98/39446 "70 Human Secreted Proteins"

**[0334]** WO 98/37418 "Compounds for Immunodiagnosis of Prostate Cancer and Methods For Their Use"

**[0335]** WO 96/03383 "Indolizine $_{s}PLA_2$ Inhibitors"

**[0336]** WO 95/14772 "Gene Signature"

**[0337]** WO 94/03599"Human cDNA and Protein Which Said cDNA Codes For"

***

**[0338]** Although the present invention has been described in detail with reference to specific embodiments, those of skill in the art will recognize that modifications and improvements are within the scope and spirit of the invention, as set forth in the claims which follow. All publications and patent documents cited herein are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference. Citation of publications and patent documents (patents, published patent applications, and unpublished patent applications) is not intended as an admission that any such document is pertinent prior art, nor does it constitute any admission as to the contents or date of the same. The invention having now been described by way of written description, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the foregoing description is for purposes of illustration and not limitation of the following claims.

WHAT IS CLAIMED IS:

1. A method of treating a human subject to reduce the likelihood of developing prostate cancer comprising

(a) identifying the subject as being in a prostate cancer susceptibility population, and

(b) administering a prophylactically effective amount of lonidamine (LND) or a lonidamine analog (LNDA) to the subject.

2. The method of claim 1, wherein the subject is diagnosed with prostatic intraepithelial neoplasia (PIN).

3. The method of claim 1, wherein the subject has an elevated serum prostate specific antigen (PSA) level.

4. The method of claim 1, wherein the subject has a rising PSA level.

5. The method of claim 1, wherein the subject has a family history of prostate cancer.

6. The method of claim 1, wherein the subject has an elevated level of a prostate cancer susceptibility marker.

7. The method of claim 1 wherein the subject has undergone a prostate biopsy in which no evidence of prostate cancer was detected.

8. A method for preventing prostate cancer in a human subject comprising administering a therapeutically effective amount of an energolytic agent (EA) to said human subject in need of such treatment, wherein the energolytic agent is an agent that interferes with energy metabolism in prostate epithelial cells.

9. The method of claim 8 wherein the energolytic agent is selected from the group consisting of 2-deoxyglucose, 3-bromopyruvate, gossypol, oxamate, iodoacetate, apoptolidin, and a londamine analog.

10. A method for preventing an epithelial cancer other than prostate cancer in a human subject comprising administering lonidamine or a lonidamine analog to a subject in need of prophylaxis.

11. The method of claim 10 comprising administering lonidamine analog to the subject.

FIGURE 1

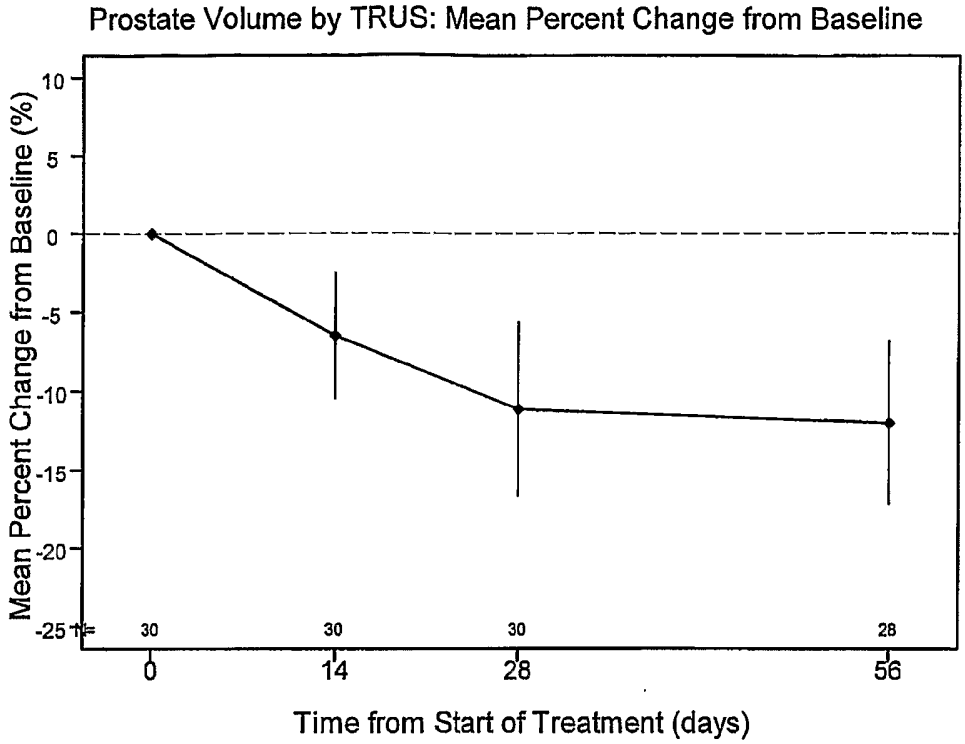
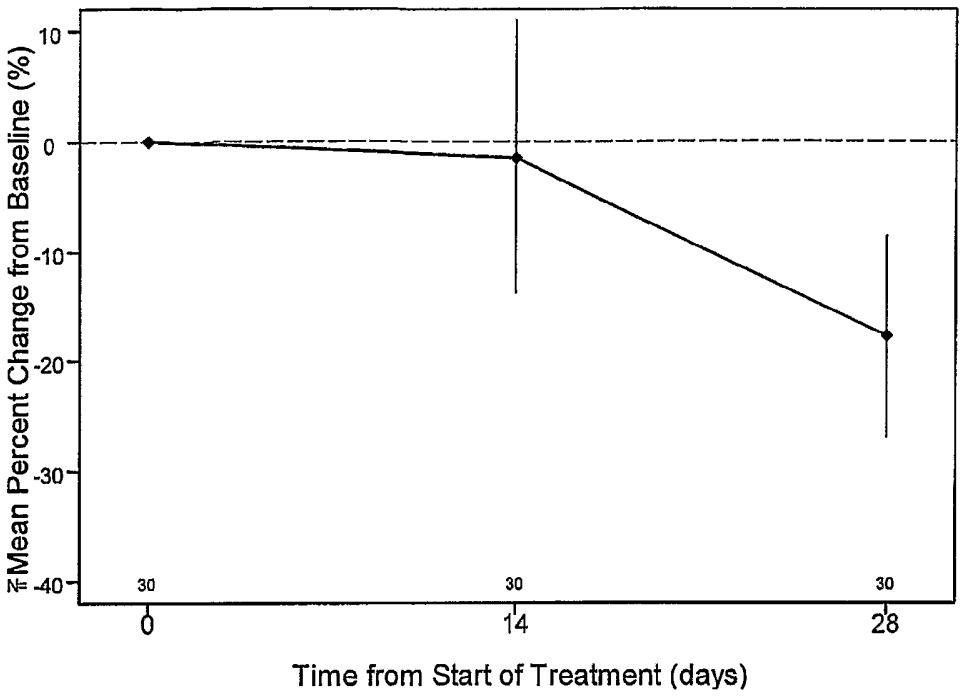

Prostate Volume by TRUS: Mean Percent Change from Baseline
Mean Percent Change from Baseline (%)
10
5
0
-5
-10
-15
-20
-25
n=
30
30
30
28
0
14
28
56
Time from Start of Treatment (days)

FIGURE 2

PSA: Mean Percent Change from Baseline (with 95% Confidence Intervals)



# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/24423

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 31/415
US CL : 514/403
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)
U.S. : 514/415

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
none

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | DUDAK et al. Enhancement of Radiation Response of Prostatic Carcinoma by Lonidamine, Anticancer Research. 1996, Vol. 16, No. 6B, pages 3665-3672, especially pages 3667-3668. | 1-7 |
| X<br>---<br>Y | RAVAGNAN et al. Lonidamine triggers apoptosis via direct, Bcl-2-inhibited effect on the mitochondrial permeability transition pore, Oncogene. 1999, Vol. 18, pages 2537-2546, especially pages 25-37-2538. | 10-11<br>---<br>1-7 |
| X | US 5,260,327 A (KIM et al.) 09 November 1993 (09.11.1993), see entire document, especially column 2, lines 33-40; column 3, lines 58-66. | 8-9 |
| X | US 6,114,397 A (FLACK et al.) 05 September 2000 (05.09.2000), see entire document, especially column 1, lines 45-63; column 7, lines 29-34. | 8-9 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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|---|---|
| 30 September 2005 (30.09.2005) | 04 NOV 2005 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US<br>Commissioner for Patents<br>P.O. Box 1450<br>Alexandria, Virginia 22313-1450<br>Facsimile No. (571) 273-3201 | Authorized officer<br>Cybille Delacroix-Muirheid<br>Telephone No. 571-272-0572 |

Form PCT/ISA/210 (second sheet) (April 2005)

Continuation of B. FIELDS SEARCHED Item 3:
EAST: lonidamine, breast, mammary, prostate, ovarian, cancer, carcinoma, neoplasm, tumor, tumour, 2-deoxyglucose, 3-bromopyruvate, gossypol, oxamate, apoptolidin