A method of treatment and/or prevention of cancer comprises administering agents which cause increased intracellular granularity in cancer cells, at least in an amount sufficient to inhibit proliferation of such cells and preferably in an amount sufficient to lead to cancer cell death. The method is particularly directed to refractory cancer, particularly hormone refractory prostate cancer. The agents identified cause increased intracellular granularity in the cancer cells, and also convert adherent cancer cells to non-adherent cancer cells, leading to cancer cell death. Using the present invention, cancer cells undergo increased intracellular granularity at relatively low agent concentrations, while also inhibiting cell proliferation. Increased concentrations lead to conversion of adherent cancer cells to non-adherent cancer cells, then to cell death. While the exact mechanism of cancer cell degradation and death is not completely understood, the treated cancer cells, including refractory prostate cancer cells, give indications of cell death through an autophagic mechanism. Pharmaceutical compositions related to the presently disclosed methods are also disclosed.
Field of the Invention

This invention relates to a method for preventing and/or treating cancer using therapeutic compositions containing compounds which induce increased cellular granularity, and conversion of cancer cells from adherent to non-adherent, leading to cancer cell death.

Federally Sponsored Research

The present invention was made with government support under Grant No. 1U54MH074425-01P1, awarded by the National Institute of Health. As a result, the Government has certain rights in the invention.

Cross Reference to Related Applications

This application claims priority in U.S. Provisional Patent Application no. 61/131,015, filed June 5, 2008, which is incorporated by reference in its entirety herein.

Background of the Invention

Cancer is a multifaceted disease characterized by an increase in the number of abnormal cells derived from a given normal tissue, with these cells typically invading adjacent tissues, or metastasizing by spreading through the blood to other regions of the body. Cancer typically progresses through a multistep process that begins with minor preneoplastic changes, which may progress to neoplasia, the neoplastic lesions possibly developing an increasing capacity for invasion, growth, metastasis, and heterogeneity.

There is an enormous variety of cancers, with examples including cancer of the lung, colon, rectum, prostate, breast, brain, and intestine. The incidence of cancer continues to climb as the general population ages, as new cancers develop; and as susceptible populations grow. A tremendous demand exists for new methods and compositions that can be used to treat patients with cancer.

As a one specific example, most men will in their lifetime experience problems that
stem from diseases in their prostate. Malignancies of the prostate gland are the most common form of cancer to occur among men in the United States, affecting over 180,000 men.

Current therapy may involve surgery, chemotherapy, hormonal therapy and/or radiation treatment to eradicate neoplastic cells in a patient. Other therapies involve biological therapy or immunotherapy. All of these approaches pose significant drawbacks for the patient. Surgery, for example, may be contraindicated due to the health of a patient or may be unacceptable to the patient. Additionally, surgery may not completely remove neoplastic tissue. Radiation therapy is only effective when the neoplastic tissue exhibits a higher sensitivity to radiation than normal tissue. Radiation therapy can also often elicit serious side effects. Hormonal therapy is rarely given as a single agent. Although hormonal therapy can be effective, it is often used to prevent or delay recurrence of cancer after other treatments have removed the majority of cancer cells. Biological therapies and immunotherapies are limited in number and may produce side effects such as rashes or swellings, flu-like symptoms, including fever, chills and fatigue, digestive tract problems or allergic reactions.

When prostate cancer is diagnosed prior to metastasis, the patient has a greater than 99% chance of survival. The most successful means for treating prostate cancer at this stage is a radical prostatectomy. Unfortunately, this surgery carries with it the risk of severing nerves and blood vessels associated with sexual organs and the bladder, and can potentially result in impotency or incontinency. Radiation therapy is yet another commonly used procedure that carries the risk of impotency. Half the patients who undergo radiation therapy for prostate cancer become impotent within 2 years of treatment. In addition to the adverse affects associated with these procedures, they are significantly less effective in patients whose cancer has already delocalized or metastasized on diagnosis. In these cases, patients generally undergo even more invasive procedures such as hormonal therapy or chemotherapy. Unfortunately, most patients eventually stop responding to hormonal therapy and the most successful chemotherapeutic, Taxotere, only prolongs the life of advanced prostate cancer patients by 2.5 months on average.

As another alternative therapeutic, monoclonal antibody (mAb)-based immunotherapy has proven clinically beneficial for cancer patients while allowing them to maintain a good quality of life. These antibodies can either regulate proliferation of cancer cells through the manipulation of signal transduction, or promote cytotoxicity. Two examples of FDA-
approved mAb-based anticancer drugs are Herceptin and Rituxan (Rituximab), which are currently being used for the treatment of breast cancer and non-Hodgkin’s lymphoma, respectively. While there are no mAb-based therapeutics currently available for prostate cancer patients, advanced clinical studies on mAb-based immunotherapy has shown promise for the treatment of prostate cancer including advanced prostate cancer. Despite the major advantages of mAb-based immunotherapy, there are significant pitfalls which may limit its potential. In general, mAb-based therapeutics are highly costly ($70,000 for full course of treatment of Herceptin), lack oral bioavailability, and can lead to severe and often fatal side-effects. For example, Herceptin is associated with heart problems and cannot be administered to approximately 10% of cancer patients because of heart-related complications. Rituxan can cause several side-effects which include renal failure, infections and immune and pulmonary toxicity.

Overall, chemotherapy often has many drawbacks. Many chemotherapeutic agents are toxic to healthy cells, and chemotherapy can cause significant and dangerous side effects, including severe nausea, bone marrow depression, and immunosuppression. Additionally, even with administration of combinations of chemotherapeutic agents, many tumor cells are resistant or develop resistance to the chemotherapeutic agents. In fact, those cells resistant to the particular chemotherapeutic agents used in the treatment protocol often prove to be resistant to other drugs, even if those agents act by a different mechanism from the drugs used in the specific treatment. This phenomenon is referred to as pleiotropic drug or multidrug resistance. Because of such drug resistance, many cancers prove refractory to standard chemotherapeutic treatment protocols.

For example, while treatments for prostate cancer do exist, over time prostate cancer can become refractory to the treatments, and once refractory, there are few options available for treating advanced, hormone refractory prostate cancer. Consequently, it is of vital importance to identify new methods of cancer prevention and treatment, particularly against refractory cancer cells.

**Summary Of The Invention**

It is an object of the present invention to identify compounds that are useful in the treatment of cancer.

It is a further object to provide methods of treatment and/or prevention of cancer comprising administering agents which cause increased intracellular granularity in cancer
cells, at least in an amount sufficient to inhibit proliferation of such cells and preferably in an amount sufficient to inhibit proliferation of such cells and preferably in an amount sufficient to lead to cancer cell death.

It is yet another object to provide a method of treatment for cancer, including refractory cancer, particularly prostate cancer including hormone refractory prostate cancer, as well as metastatic prostate cancer by administering agents which cause increased intracellular granularity in the refractory cancer cells, at least in an amount sufficient to inhibit proliferation of such cells and preferably in an amount sufficient to lead to refractory cancer cell death. This method may optionally utilize radiation therapy to treat cancer in combination with the agents of the present invention, which also exhibit radiosensitizing activity.

It is another object to provide a method for treating cancer, including refractory cancer, particularly prostate cancer including hormone refractory prostate cancer, as well as metastatic prostate cancer by administering agents which cause increased intracellular granularity in the refractory cancer cells, at least in an amount sufficient to inhibit proliferation of such cells and preferably in an amount sufficient to lead to refractory cancer cell death, in combination with one or more secondary anticancer agents or other agents which are effective in treating cancer, especially prostate cancer, including metastatic prostate cancer as otherwise described herein and further in combination with a pharmaceutically acceptable carrier, additive or excipient. Optionally, radiation therapy may be combined with the anticancer included in this method to take advantage of the radiosensitizer activity of anticancer agents of the present invention.

It is still another object of the invention to provide pharmaceutical compositions which comprise primary anticancer agents which cause increased intracellular granularity in cancer cells, especially refractory cancer cells, as otherwise disclosed herein in combination with a secondary anticancer agent or other agent which is found to be useful in treating cancer, especially prostate cancer, including metastatic prostate cancer as otherwise described herein and further in combination with a pharmaceutically acceptable carrier, additive or excipient.

Without being limited by way of theory, these and other objects of the present invention are achieved by a method which comprises administering to a mammal an effective amount of a composition as otherwise described herein sufficient for increasing the intracellular granularity of cancer cells, thereby inhibiting cell proliferation and preferably in an amount sufficient to cause cancer cell death. In addition, the inventive method further
comprises administering a composition in an amount effective for converting adherent cancer cells to non-adherent cancer cells, leading to cancer cell death.

Using the present invention, cancer cells undergo increased intracellular granularity at relatively low concentrations of the inventive compositions, while also inhibiting cell proliferation. Increased concentrations lead to conversion of adherent cancer cells to non-adherent cancer cells, then to cell death. While the exact mechanism of cancer cell degradation and death is not completely understood, the treated cancer cells, including refractory prostate cancer cells, do not produce indications of apoptotic cell death, but rather give indications of cell death through an autophagic mechanism.

**Brief Description Of The Drawings**

Figure 1 shows the chemical structure of astemizole (AST), one of the compounds found useful in accordance with the present invention.

Figures 2A and 2B are flow cytometric analyses of prostate cancer cells used to measure levels of intracellular granularity using side scatter (SSC-H) as an endpoint. Untreated, control cells are shown in Fig. 2A with low SSC-H levels present in the gated region R1, whereas high intra-cellular granularity is seen in treated cells as shown in Fig. 2B.

Figure 3 shows 200X phase contrast microscopy images showing increased intracellular granularity of treated PC3 human prostate cancer cells.

Figure 4 shows a dose-response for induction of intracellular granularity followed by prostate cancer cell death. Cell death is shown circled at 10 µM.

Figure 5 shows cell death measured in CWRv22 and LAPC4 human prostate cancer cell lines using trypan blue uptake and hemacytometry. SCC cells are a skin cancer cell line that were not killed by treatment with AST up to 15 µM.

Figure 6 shows cell death measured in PC3 androgen-independent human prostate cancer cells using trypsin blue uptake and hemacytometry.

Figure 7 shows a cell growth analysis in multiple prostate cancer cell lines treated with astemizole, Fig. 7A with DDU 45 cells, Fig. 7B with PC3 cells, Fig. 7C with LAPC4 cells, and Fig. 7D with LNCaP cells.

Figure 8 shows the induction of an LC3 II band in CWRv22 cells by 10 µM astemizole, indicating that autophagy is induced in these cells.
Detailed Description Of The Invention

The term "cancer" is used throughout the specification to refer to the pathological process that results in the formation and growth of a cancerous or malignant neoplasm, i.e., abnormal tissue that grows by cellular proliferation, often more rapidly than normal and continues to grow after the stimuli that initiated the new growth cease. Malignant neoplasms show partial or complete lack of structural organization and functional coordination with the normal tissue and most invade surrounding tissues, metastasize to several sites, and are likely to recur after attempted removal and to cause the death of the patient unless adequately treated.

As used herein, the term "neoplasia" is used to describe all cancerous disease states and embraces or encompasses the pathological process associated with malignant hematogenous, ascitic and solid tumors. Representative cancers include, for example, stomach, colon, rectal, liver, pancreatic, lung, breast, cervix uteri, corpus uteri, ovary, prostate, including metastatic prostate cancer, testis, bladder, renal, brain/CNS, head and neck, throat, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, leukemia, melanoma skin cancer, non-melanoma skin cancer, acute lymphocytic leukemia, acute myelogenous leukemia, Ewing's sarcoma, small cell lung cancer, choriocarcinoma, rhabdomyosarcoma, Wilms' tumor, neuroblastoma, hairy cell leukemia, mouth/pharynx, oesophagus, larynx, kidney cancer and lymphoma, among others, which may be treated using the method of the invention, administering one or more compounds in accordance with the present invention.

The following terms are used to describe the present invention. In instances where a term is not specifically defined herein, that term is given an art-recognized meaning by those of ordinary skill applying that term in context to its use in describing the present invention.

The term "compound" (also "agent"), as used herein, unless otherwise indicated, refers to any specific chemical compound disclosed herein and includes tautomers, regioisomers, geometric isomers, and where applicable, optical isomers (enantiomers) thereof, as well as pharmaceutically acceptable salts and alternative pharmaceutically acceptable salts (i.e., a pharmaceutically acceptable salt form alternative to a pharmaceutically acceptable salt described) and derivatives (including prodrug forms) thereof. Within its use in context, the term compound generally refers to a single compound, but also may include other compounds such as stereoisomers, regioisomers and/or optical isomers (including racemic mixtures) as well as specific enantiomers or enantiomerically enriched mixtures of disclosed compounds. The term also refers, in context to prodrug
forms of compounds which have been modified to facilitate the administration and delivery of compounds to a site of activity. It is noted that in describing the present compounds, numerous substituents, linkers and connector molecules and variables associated with same, among others, are described. It is understood by those of ordinary skill that molecules which are described herein are stable compounds as generally described hereunder.

The term "patient" or "subject" is used throughout the specification within context to describe an animal, generally a mammal and preferably a human, to whom treatment, including prophylactic treatment (prophylaxis), with the compositions according to the present invention is provided. For treatment of those infections, conditions or disease states which are specific for a specific animal such as a human patient or a patient of a particular gender, such as a human male patient, the term patient refers to that specific animal. Compounds according to the present invention are useful for the treatment of cancer, especially including prostate cancer and in particular, hormone-refractory prostate cancer.

The term "effective" is used herein, unless otherwise indicated, to describe an amount of a compound or composition which, in context, is used to produce or effect an intended result, whether that result relates to the inhibition of the effects of a toxicant on a subject or the treatment of a subject for secondary conditions, disease states or manifestations of exposure to toxicants as otherwise described herein. This term subsumes all other effective amount or effective concentration terms (including the term "therapeutically effective") which are otherwise described in the present application.

The terms "treat", "treating", and "treatment", etc., as used herein, refer to any action providing a benefit to a patient at risk for cancer or refractory cancer, including improvement in the condition through lessening or suppression of at least one symptom, inhibition of cancer growth, reduction in cancer cells or tissue, prevention or delay in progression of metastasis of the cancer, prevention or delay in the onset of disease states or conditions which occur secondary to cancer or remission or cure of the cancer among others.

"Treatment", as used herein, encompasses both prophylactic and therapeutic treatment. The term "prophylactic" when used, means to reduce the likelihood of an occurrence or the severity of an occurrence within the context of the treatment of cancer, including cancer metastasis as otherwise described hereinabove.

In certain particular aspects of the present invention, the cancer which is treated is prostate cancer or hormone refractory prostate cancer. It is understood that metastatic cancer may be found in virtually all tissues of a cancer patient in late stages of the disease, for example, metastatic prostate cancer is found in seminal vesicles, lymph system/nodes
(lymphoma), in bones, in bladder tissue, in kidney tissue, liver tissue and in virtually any tissue, including brain (brain cancer/tumor). Thus, the present invention is generally applicable and may be used to treat any cancer in any tissue, regardless of etiology.

The term "prostate cancer" is used to describe a disease in which cancer develops in the prostate, a gland in the male reproductive system. It occurs when cells of the prostate mutate and begin to multiply uncontrollably. These cells may metastasize (metastatic prostate cancer) from the prostate to virtually any other part of the body, particularly the bones and lymph nodes, but the kidney, bladder and even the brain, among other tissues. Prostate cancer may cause pain, difficulty in urinating, problems during sexual intercourse, erectile dysfunction. Other symptoms can potentially develop during later stages of the disease.

For this application, the treatment of hormone-sensitive and hormone refractory prostate cancer cells in culture are used as examples, these examples providing evidence sufficient to support use in the treatment of other cancers using the method described herein, and further using these agents for chemoprevention, which includes treatment for persons in high risk groups, such as those genetically prone to develop cancer and persons that have been exposed to conditions that may promote cancer development, neo-adjuvant and adjuvant chemotherapy for cancer, and treatment of all stages of cancer after diagnosis. The method includes use of the identified agents alone as well as in combination with each other. Also, the method allows for these agents to be administered in combination with, or as supplements to, other medical treatments. For example, they may be used in combination with radiotherapy to increase the efficiency of cancer treatment.

The use of specific chemotherapeutic regimens for cancer can depend on the stage of cancer at the time the disease is diagnosed or the state to which the disease has progressed. For example, the initial chemotherapeutic treatment of prostate cancer is often performed using drugs that inhibit androgen production. After 2 to 3 years, prostate cancer becomes refractory to anti-androgen therapy necessitating the use of drugs that non-specifically attack prostate cancer. However, the current treatments for advanced, hormone-refractory prostate cancer do not greatly increase life-span and their actions are largely palliative. Therefore, novel, effective treatments for many cancers, including refractory prostate cancer, are desperately needed.

The term "radiation therapy" is used to describe therapy for prostate cancer which may be used in conjunction with the present compounds which exhibit activity as radiation
sensitizers, in addition to their inherent anticancer activity. Radiation therapy uses high doses of radiation, such as X-rays, to destroy cancer cells. The radiation damages the genetic material of the cells so that they can't grow. Although radiation damages normal cells as well as cancer cells, the normal cells can repair themselves and function, while the cancer cells cannot.

Radiation therapy may be in combination with the presently claimed compounds, which can function as radiation sensitizers or the therapy (radiation plus compounds of the present invention) may be further combined with hormonal treatment and other treatment as otherwise disclosed herein to treat prostate cancer. Radiation therapy most effective in treating cancers that have not spread outside the prostate. But it also may be used if the cancer has spread to nearby tissue. Radiation is sometimes used after surgery to destroy any remaining cancer cells and to relieve pain from metastatic cancer.

Radiation is delivered in one of two ways. External-beam radiation therapy and brachytherapy. External-beam radiation therapy uses a large machine to aim a beam of radiation at the tumor. After the area of cancer is identified, an ink tattoo no bigger than a pencil tip is placed on the skin of the subject so that the radiation beam can be aimed at the same spot for each treatment. This helps focus the beam on the cancer to protect nearby healthy tissue from the radiation. External radiation treatments usually are done 5 days a week for 4 to 8 weeks. If cancer has spread to the patient's bones, shorter periods of treatment may be given to specific areas to relieve pain.

There are basically three types of external radiation therapy: conformal radiotherapy (3D-CRT), intensity-modulation radiation therapy (IMRT) and proton therapy. Conformal radiotherapy uses a three-dimensional planning system to target a strong dose of radiation to the prostate cancer. This helps to protect healthy tissue from radiation. Intensity-modulated radiation therapy uses a carefully adjusted amount of radiation. This protects healthy tissues more than conformal radiotherapy does. Proton therapy uses a different type of energy (protons) than X-rays. This approach allows a higher amount of specifically directed radiation, which protects nearby healthy tissues the most, especially the rectum. Sometimes proton therapy is combined with X-ray therapy.

Brachytherapy, or internal radiation therapy, uses dozens of tiny seeds that contain radioactive material. It may be used preferably to treat early-stage prostate cancer. Needles are used to insert the seeds through your skin into your prostate. The surgeon uses ultrasound to locate your prostate and guide the needles. As the needles are pulled out, the seeds are left in place. The seeds release radiation for weeks or months, after which they are no longer
radioactive. The radiation in the seeds can't be aimed as accurately as external beams, but they are less likely to damage normal tissue. After the seeds have lost their radioactivity, they become harmless and can stay in place.

Radiation therapy may combine brachytherapy with low-dose external radiation. In other cases, treatment combines surgery with external radiation. In the present invention, compounds which are otherwise claimed may be used as radiation sensitzers.

The term "coadministration" shall mean that at least two compounds or compositions are administered to the patient at the same time, such that effective amounts or concentrations of each of the two or more compounds may be found in the patient at a given point in time. Although compounds according to the present invention may be co-administered to a patient at the same time, the term embraces both administration of two or more agents at the same time or at different times, provided that effective concentrations of all coadministered compounds or compositions are found in the subject at a given time. Anticancer compounds according to the present invention (primary anticancer agents) may be administered with one or more additional (secondary) anti-cancer agents or other agents which are used to treat or ameliorate the symptoms of cancer, especially prostate cancer, including metastatic prostate cancer. Exemplary anticancer agents which may be coadministered in combination with one or more chimeric compounds according to the present invention include, for example, antimetabolites, inhibitors of topoisomerase I and II, alkylating agents and microtubule inhibitors (e.g., taxol). Specific anticancer compounds for use in the present invention include, for example, aldesleukin; alemtuzumab; alitretinoin; allopurinol; altretamine; amifostine; anastrozole; arsenic trioxide; asparaginase; BCG Live; bexarotene capsules; bexarotene gel; bleomycin; busulfan intravenous; busulfan oral; calusterone; capecitabine; carboplatin; carmustine; carmustine with Polifeprosan 20 Implant; celecoxib; chlorambucil; cisplatin; cladribine; cyclophosphamide; cvtarabine; cvtarabine liposomal; dacarbazine; dactinomycin; actinomycin D; Darbepoetin alfa; daunorubicin liposomal; daunorubicin; daunomycin; Denileukin diftitox; dexrazoxane; docetaxel; doxorubicin; doxorubicin liposomal; Dromostanolone propionate; Elliott's B Solution; epirubicin; Epoeitin alfa; estramustine; etoposide phosphate; etoposide (VP-16); exemestane; Filgrastim; floxuridine (intraarterial); fludarabine; fluorouracil (5-FU); fulvestrant; gemtuzumab ozogamicin; goserelin acetate; hydroxyurea; Ibritumomab Tiuxetan; idarubicin; ifosfamide; imatinib mesylate; Interferon alfa-2a; Interferon alfa-2b; irinotecan; letrozole; leucovorin; levamisole; lomustine (CCNU); meclorethamine (nitrogen mustard); megestrol acetate; melphalan (L-PAM); mercaptopurine (6-MP); mesna; methotrexate; methoxsalen; mitomycin C; mitotane;
In addition to anticancer agents, a number of other agents may be coadministered with primary anticancer compounds according to the present invention in the treatment of cancer, especially prostate cancer, including metastatic prostate cancer. These include active agents, minerals, vitamins and nutritional supplements which have shown some efficacy in inhibiting prostate cancer tissue or its growth or are otherwise useful in the treatment of prostate cancer. For example, one or more of dietary selenium, vitamin E, lycopene, soy foods, vitamin D, green tea (e.g., extract), omega-3 fatty acids and-phytoestrogens, including beta-sitosterol,-- may be utilized in combination with the present compounds to treat prostate cancer, especially refractory prostate cancer and metastatic prostate cancer.

In addition, active agents, other than traditional anticancer agents have shown some utility in treating prostate cancer. The selective estrogen receptor modulator drug toremifene may be used in combination with the present compounds to treat cancer, especially prostate cancer, including metastatic prostate cancer. In addition, two medications which block the conversion of testosterone to dihydrotestosterone, finasteride and dutasteride, are also useful in the treatment of prostate cancer when coadministered with compounds according to the present invention. The phytochemicals indole-3-carbinol and diindolylmethane may also be coadministered with the present compounds for their effects in treating prostate cancer.

Additional agents which may be combined with compounds according to the present invention include antiandrogens, for example, flutamide, bicalutamide, nilutamide, and-- cyproterone acetate as well as agents which reduce the production of adrenal androgens (e.g. DHEA), such as ketoconazole and aminoglutethimide. Other active agents which may be combined with compounds according to the present invention include, for example, GnRH modulators, including agonists and antagonists. GnRH antagonists suppress the production of LH directly, while GnRH agonists suppress LH through the process of downregulation after an initial stimulation effect. Abarelix is an example of a GnRH antagonist, while the
GnRH agonists include leuprolide, goserelin, triptorelin, and buserelin, among others. These agents may be combined with compounds according to the present invention in effective amounts. In addition, abiraterone acetate may also be combined with one or more compounds according to the present invention in the treatment of prostate cancer, especially including metastatic prostate cancer.

Other agents which may be combined with one or more compounds according to the present invention include the bisphosphonates such as zoledronic acid, which have been shown to delay skeletal complications such as fractures which occur with patients having metastatic prostate cancer. Alpharadin, another agent, may be combined with compounds according to the present invention to target bone metastasis. In addition, bone pain due to metastatic prostate cancer may be treated with opioid pain relievers such as morphine and oxycodone, among others, which may be combined with compounds according to the present invention.

Coadministration may also refer to the administration of radiation therapy in combination with one or more compounds according to the present invention which may also serve as radiation sensitizers in addition to anticancer agents, optionally in combination with an addition anticancer agent or other agent as described herein which is useful for treating prostate cancer.

The ability of the agents identified herein, for use in the method of the invention, to kill cancer cells was determined by the ability of the agents to induce intracellular granularity. To illustrate the utility of these agents, prostate cancer cell lines were used under conditions that were optimal for induction of markers of intracellular, secretory granules. The identification of agents that induce intracellular granularity in cancer cells as an indicator of agents useful for cancer treatment is entirely novel. High-throughput methods were integrated to efficiently identify chemicals that induce intracellular granularity in prostate cancer cell lines.

Using these methods, the Prestwick chemical library was screened for agents that lead to an increase in intracellular granularity of prostate cancer cells. In addition to detecting increases in intracellular granularity, this methodology detects cell death by identifying chemicals that cause the adherent cells in this assay to become non-adherent. Multiple chemicals have been identified using this technology that are believed to be useful for the prevention and treatment of cancer.
Table 1 includes those chemicals that increase intracellular granularity leading to cancer cell death. It was determined that after reaching peak concentrations of these agents for producing intracellular granularity, these chemicals can lead to cancer cell death. Low doses of these agents cause increased intracellular granularity, which ultimately lead cells to become non-adherent, reducing cell numbers because of cancer cell death (see Fig. 3). For each of these groups, compounds have been selected to test to confirm their efficacy. These compounds may be combined for greater efficacy.

**Table I** - compounds that increase intracellular granularity and which cause cancer cell death (primary anti-cancer agents according to the present invention):

<table>
<thead>
<tr>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-hydroxymethyl-beta-carboline</td>
</tr>
<tr>
<td>Albendazole</td>
</tr>
<tr>
<td>Alprenolol hydrochloride</td>
</tr>
<tr>
<td>Ambroxol hydrochloride</td>
</tr>
<tr>
<td>Amitryptiline hydrochloride</td>
</tr>
<tr>
<td>Amodiaquin dihydrochloride dihydrate</td>
</tr>
<tr>
<td>Amoxapine</td>
</tr>
<tr>
<td>Antazoline hydrochloride</td>
</tr>
<tr>
<td>Anisomycin</td>
</tr>
<tr>
<td>Antimycin A</td>
</tr>
<tr>
<td>Apomorphine hydrochloride</td>
</tr>
<tr>
<td>Astemizole</td>
</tr>
<tr>
<td>Azacyclonol</td>
</tr>
<tr>
<td>Benperidol</td>
</tr>
<tr>
<td>Benzethonium chloride</td>
</tr>
<tr>
<td>Benzydamine hydrochloride</td>
</tr>
<tr>
<td>Bepridil hydrochloride</td>
</tr>
<tr>
<td>Bromperidol</td>
</tr>
<tr>
<td>Cephaeline dihydrochloride heptahydrate</td>
</tr>
<tr>
<td>Chelidonine monohydrate</td>
</tr>
<tr>
<td>Chloropyramine hydrochloride</td>
</tr>
<tr>
<td>Chlorotrianisene</td>
</tr>
<tr>
<td>Chlorpromazine hydrochloride</td>
</tr>
<tr>
<td>Chlorprothixene hydrochloride</td>
</tr>
</tbody>
</table>
Chrysene-1, 4-quinone
Clomiphene citrate
Clomipramine hydrochloride
Corticosterone
Cyclobenzaprine hydrochloride
Cyproheptadine hydrochloride
Diaziquone
Dibucaine
Dienestrol
Digitoxigenin
Digoxin
Dihydroergocristine mesylate
Dihydroergotoxine mesylate
Domperidone
Doxepin hydrochloride
Emetine dihydrochloride
Epiandrosterone
Ethisterone
Fenbendazole
Fendiline hydrochloride
Fluoxetine hydrochloride
Fluphenazine dihydrochloride
GBR 12909 dihydrochloride
Gossypol
Gramicidin
7-benzyloxy Gramine
Halopropin
Harmaline hydrochloride dehydrate
Hycanthone
Ifenprodil tartrate
Imipramine hydrochloride
Ketotifen fumarate
Lanatoside C
Lidoflazine
Loperamide hydrochloride
Maprotiline hydrochloride
Mebendazole
Mebhydroline 1,5-naphtalenesulfonate
Menadione
Mesoridazine besylate
Metergoline
Methimazole
Methiothepin maleate
Methotrimemazine maleate salt
Metixene hydrochloride
Mycophenolic acid
Niclosamide
Nitrarine dihydrochloride
Nocodazole
Norcyclobenzaprine
Norethynodrel
Nortriptyline hydrochloride
Parthenolide
Perphenazine
Pemexiline maleate
Pimozide
Piperlongumine
Phenoformin hydrochloride
Primaquine diphosphate
Prochlorperazine dimaleate
Propafenone hydrochloride
Pyrimethamine
Quinacrine dihydrochloride dihydrate
Quinidine hydrochloride monohydrate
Remerine hydrochloride
Rescinnamin
Securinine
Scoulerine
Solanine alpha
Strophanthidin
Suloctidil
Tacrine hydrochloride hydrate
Terconazole
Terfenadine
Thimerosal
Thioridazine hydrochloride
1-phenyl-3-(2-thiazolyl)-2-Thiourea
Triamterene
Trifluoperazine dihydrochloride
Triflupromazine hydrochloride
dihydrochloride
Trimeprazine tartrate
Trimipramine maleate
Ursolic acid
Zimelidine dihydrochloride monohydrate

One chemical, astemizole (AST), was chosen for more detailed study. AST has the KJPAC name \textit{l-[(4-fluorophenyl)methyl]-N-[1-[2-(4-ethoxyphenyl)ethyl]-4-piperidyl]benzoimidazol-2-amine}, CAS number 68844-77-9, chemical formula $\text{C}_{28}\text{H}_{31}\text{FN}_{4}\text{O}$, mol. mass 458.571. It was discovered that human cancer cells, as for example prostate cancer cells, cultured with AST at concentrations above 5 $\mu$M undergo rapid cell death. Previous reports have described the therapeutic value of AST, but there are no reports of therapeutic value related to cancer treatment.

For example, it is known that AST acts as a histamine H1-receptor antagonist with a long duration of action. In this regard, AST acts to suppress actions mediated by histamine, such as edema and pruritus manifesting anticholinergic and antipruritic effects. AST competitively binds to histamine H1-receptor sites in the gastrointestinal tract, uterus, blood vessels, and bronchial muscle. Pharmacokinetically, AST is rapidly absorbed from the gastrointestinal tract; protein binding is around 96%. AST does not cross the blood-brain barrier and H1 receptor binding is mostly in the peripheral rather than central nervous system. There is nothing which would lead one to believe that AST could be an effective agent for the treatment of cancer.
Materials and Methods

Chemicals: Initial screens were performed using chemicals present in the Prestwick Chemical Library (Illkirch, France). Follow up studies, including those performed with astemizole (Sigma-Aldrich Cat. No. A6424), used chemicals acquired from Sigma-Aldrich Chemical Co. (St. Louis, MO), unless specified otherwise. The chemical structure of astemizole is shown in Fig. 1.

Cell Culture: The androgen-sensitive CWRv22 (i.e., 22Rv1) and LNCaP as well as the androgen-independent DU145 and PC3 human prostate cancer cell lines were acquired from American Type Culture Collection (Manassas, VA). All cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA) containing 5% heat-inactivated fetal calf serum (FCS; Sigma, St. Louis, MO) with streptomycin-penicillin antibiotics (designated DMEM/FCS) in a 5% CO₂ incubator at 37°C. LAPC4 cells adapted to growth in DMEM and 5% FCS were acquired from George Wilding (University of Wisconsin, Paul P. Carbone Comprehensive Cancer Center).

Cell Proliferation Assay: To determine the number of proliferating cells after astemizole treatment, cells were seeded in 96-well tissue culture plates at 5,000 cells per well in 100 µL of DMEM/FCS. Two days after plating, cells were treated with astemizole at the indicated concentrations. Four days after treatment, cell proliferation was determined using the CyQUANT NF Cell Proliferation Assay Kit (Invitrogen, Carlsbad, CA) according to the kit instructions.

Cell Viability Assays: Two assays were used to determine cell viability: propidium iodide exclusion with flow cytometry and trypan blue exclusion-with hemacytometry. -Cells were treated with astemizole at the indicated concentrations for 24 to 96 hours prior to viability analysis. For propidium iodide cell viability determinations, cells were removed from the dishes with 0.05% trypsin. All cell culture medium was collected for the analysis in order to include any cells that may have released from the plate during the incubation period. Propidium iodide was added at a final concentration of 2 µg/ml and cells were analyzed on a Becton Dickinson FACScan flow cytometer. For trypan blue dye exclusion studies, all cell culture medium and cells removed by trypsinization were collected as above. An aliquot of these cells was re-suspended in 0.4% trypan blue. Both viable and non-viable cell counts were obtained by manual counting using a hemacytometer and light microscopy.
HyperCyt and High-throughput Data Analysis: The HyperCyt® cytometry analysis system interfaces a flow cytometer and an autosampler. While the sampling probe moves from well to well, a peristaltic pump sequentially aspirates a small volume from each well. A bubble of air, created by the continuously running pump, separates samples from individual wells. Sample and bubble volumes are determined by the time the sampling probe is in a well (sip time) or in taking air while moving from well to well (up time). HyperCyt® sampling of a single 384-well plate is completed in less than 15 minutes. This system has been validated using cell-based high-throughput endpoint assays for ligand binding, surface antigen expression, and immunophenotyping.

The air-bubble separated samples, collected by HyperCyt®, are delivered continuously to the cytometer. The data accumulates as a single, time-resolved file with gaps corresponding to air-bubbles. Both forward and side light scattering properties of treated LNCaP cells were measured using a 488 nm laser. Time-resolved data were analyzed by proprietary software (IDLQuery) set to search for 384 separate data clusters. Data from negative (0.1% DMSO) replicate wells were pooled and used to set gates that define granularity in vehicle-treated LNCaP cells. Negative control samples were defined as an LNCaP population where 10% of the cells exhibit increased granularity based on side scatter properties. A gate is set reflecting this distinction that is then applied to each data cluster. These values are automatically exported to a Microsoft Excel spreadsheet template that calculates percent side scatter shift in individual treated samples.

Microscopic and Flow Cytometric Scatter Analysis: Light microscopy images of prostate cancer cells in culture were collected using an Olympus 1X70 inverted microscope and a 20× objective. Flow cytometric analysis of forward scatter (FSC) and side scatter (SSC) were preformed using the UNM Shared Flow Cytometry Resource on a Cytomation Mo Flo, an Epics Elite, a Becton Dickinson FACScan, or a Becton Dickinson FACS Calibur.

Results

High-throughput methods were developed using the HyperCyt system and flow cytometry to efficiently identify agents that induce cancer cells to express markers of increased intracellular granularity. To achieve this goal, flow cytometry using side-scatter (SSC) as an endpoint is a sensitive method to examine cells for levels of intracellular granularity. Astemizole (AST) was chosen as a representative agent from Table 1 to characterize more fully for the induction of intracellular granularity and cancer cell death.
Using SSC measured by flow cytometry, astemizole (AST) was found to significantly increase cancer cell intracellular granularity (Fig. 2). The increase in intracellular granularity in cancer cells can be directly observed in PC3 human prostate cancer cells by light microscopy (Fig. 3). An increase in cancer cell intracellular granularity was found to be a predictive marker of agents that ultimately result in cancer cell death.

Use of the HyperCyt flow cytometry system allows for the detection of cell death by identifying compounds that cause adherent cancer cells in the assay to become non-adherent when they die. This is illustrated in Fig. 4 showing a dose-response for AST-induced increases in intracellular granularity (i.e., up to 3 μM), which is followed by cell death seen at a dose of 10 μM. The action of AST on cancer cells to induce intracellular granularity and cell death has not previously been observed.

Cell killing is the most important endpoint for agents targeted at cancer treatment. Multiple prostate cancer cell lines were used to determine cytotoxicity of AST in culture on cancer cells. The action of AST against the androgen-sensitive LNCaP, LAPC4, and CWRv22 human prostate cancer cells lines was evaluated and found to show a dose-response to cell killing by AST treatment. For example, as shown in Fig. 5, CWRv22 and LAPC4 human prostate cancer cells were treated with 6, 10, and 15 μM AST for 24 hours and the number of non-viable cells was determined by trypan blue uptake and hemacytometry. Both CWRv22 and LAPC4 were highly sensitive to AST even at the lowest dose tested of 6 μM where 80% of the cells were dead within 24 hours of treatment. In contrast, skin cancer cells (SCC) were found to be resistant to cell death by AST treatment (Fig. 5).

In addition to inducing cell death in androgen-sensitive cell lines described above, AST was equally effective at inducing cell death in the androgen-independent PC3 cell line (Fig. 6). PC3 prostate cancer cells were evaluated for induction of cell death after treatment with AST at 5, 10, and 15 μM for 48 hours (Fig. 6) as measured by trypan blue uptake. In this study, greater than 25% cell death was observed in PC3 cells treated with 10 μM for 48 hours. Importantly, nearly 100% of PC3 cells were dead following treatment with 15 μM AST (Fig. 6). AST inhibited the growth of PC3 cells over a 4 day period at concentrations as low as 1 μM AST (Fig. 7). The significant reduction in prostate cancer cell growth induced by AST was observed in DU145, LAPC4, and LNCaP human prostate cancer cells (Fig. 7). Therefore, AST was found to be a potent inhibitor of prostate cancer cell growth among all cell lines tested and at higher concentrations, AST treatment resulted in prostate cancer cell death.
Multiple mechanisms could account for the cell death observed in cancer cells from treatment with AST. Cell death in PC3 cells occurs within 24 hours of treatment but it does not produce hallmarks of apoptotic cell death, such as the production of a hypodiploid population of cells as measured using propidium iodide incorporation and flow cytometry. Intriguingly, CWRv22 prostate cancer cells treated with 10 μM AST for 6 hours show an increase in the LC3 II band associated with autophagic pathways (Fig. 8). Thus, administering the agents identified above that induce intracellular granularity in cancer cells may produce cell death through an autophagic mechanism.

As to the use of astemizole itself as the agent selected from Table 1, pharmacokinetic studies in man demonstrated that astemizole is rapidly absorbed, peak plasma concentrations being attained within 1 to 4 hours. Astemizole is extensively metabolized, and excreted as metabolites chiefly in the feces. The half-life of the active ingredient is approximately 1 day and the elimination half-life of the active metabolites is 10 to 14 days. Astemizole was originally indicated for the treatment of allergic rhinitis, allergic conjunctivitis and chronic urticaria, though it was removed from the U.S. market in 1999. More recently, astemizole has been found to be a potent anti-malarial. The recommended administration would be in an oral form, such as by way of 10 mg tablets, one such administration being of from 1-30 mg per day, depending on the particulars of the patient to whom the dosage would be administered, more preferably being at from 5-20 mg. per day. Optimal absorption is achieved when taken on an empty stomach. Although overdoses have been reported with no ill effects, rare cases of serious life-threatening cardiovascular adverse events including QT-prolongation, torsades de pointes, and other ventricular arrhythmias have been observed in patients exceeding recommended doses of astemizole. It is believed that similar dosages of the other compounds from Table 1 can be readily determined, the dosage for astemizole being illustrative thereof.

Compounds according to the present invention may also function as radiosensitizers in addition to their anticancer activity. A study was performed showing prostate cancer cell killing actions of astemizole in combination with other therapeutic interventions. Astemizole alone produced a decrease in 22Rv1 prostate cancer cell viability with 8 μM 24 hours after treatment. In combination with 10 nM docetaxel, 2, 4, and 8 μM astemizole showed additive levels of cell killing after 24 hours. In combination with 10 gray radiation, 2, 4, and 8 μM astemizole showed synergistic levels of cell killing after 24 hours. The results are set forth in the table presented hereinbelow. These studies show that astemizole acts as a radiosensitizer
making prostate cancer cells more susceptible to the cell killing effects of ionizing radiation. This is true of the other agents which are described herein.

**TABLE 1**

<table>
<thead>
<tr>
<th>% Viability*</th>
<th>rAstemizole (uM) - Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>89.7 (2.2)</td>
</tr>
<tr>
<td>0 nM docetaxel</td>
<td>80.7 (1.7)</td>
</tr>
<tr>
<td>10 Gv radiation</td>
<td>69.3 (4.2)</td>
</tr>
</tbody>
</table>

* Viability in 22Rv1 prostate cancer cells determined 24 h after treatment by trypan blue exclusion; n = 3.

* Additive action determined with combination therapy.

* Synergistic action determined with combination therapy.

It is noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the," include plural referents unless expressly and unequivocally limited to one referent. Thus, for example, reference to "a cell" includes two or more different cells. As used herein, the term "include" and its grammatical variants are intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that can be substituted or other items that can be added to the listed items.

The method of the invention contemplates the administration of an agent which causes increased intracellular granularity in cancer cells, at least in an amount sufficient to inhibit proliferation of such cells and preferably in an amount sufficient to lead to cancer cell death, but more preferably as a pharmaceutical composition, which can be formulated using known methods to adapt the agent for administration via known routes, such as topically, orally, transdermally, rectally, vaginally, parentally, intranasally, intraocularly, intravenously, intramuscularly, intraarterially, intraorbitally, intracapsularly, intraorbitally, intracardiaclely, intradermally, intraperitoneally, transtracheally, subcutaneously, subcuticularly,
intraarticular, subcapsularly, subarachnoidly, intraspinally, intrasternally or by inhalation. Thus, the compounds according to the invention may be formulated for administration in any convenient way for use in human or veterinary medicine, and formulated into pharmaceutically acceptable dosage forms such as described below or by other conventional methods known to those of skill in the art.

[0057] The method of the invention thus includes administering pharmaceutically acceptable compositions containing a therapeutically effective amount of an agent which causes increased intracellular granularity in cancer cells, which may be one or more of the compounds described above in Table 1, formulated together with one or more pharmaceutically acceptable carriers and/or diluents. The pharmaceutical compositions may be formulated for administration in solid or liquid form, and adapted for oral administration, as aqueous or non-aqueous solutions or suspensions, tablets, boluses, powders, granules, pastes for application to the tongue or adapted for parenteral administration by subcutaneous, intramuscular or intravenous injection. Topical applications by way of a cream, ointment or spray may be of particular interest in countering the effects of toxicants, post exposure, as well as formulations that are administered via inhalation.

The phrase "therapeutically effective amount" as used herein means that amount of a compound, material, or composition which is effective for producing a desired therapeutic effect by increasing intracellular granularity in cancer cells, and/or converting adherent cancer cells to non-adherent cancer cells, leading to cancer cell death, at least in an amount sufficient to inhibit proliferation of such cells and preferably in an amount sufficient to lead to cancer cell death in at least a sub-population of cells in an animal and thereby blocking the biological consequences of that function in the treated cells, at a reasonable benefit/risk ratio applicable to any medical treatment.

"Pharmaceutically acceptable" as used herein means that the compound or composition is suitable for administration to a subject to achieve the treatments described herein, without unduly deleterious side effects in light of the severity of the disease and necessity of the treatment.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject antagonists from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient.
It should be understood that various additives, such as wetting agents, emulsifiers and lubricants, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions administered according to the present invention.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges, powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles and/or as mouth washes and the like, each containing a predetermined amount of the agent which causes increased intracellular granularity in cancer cells, at least in an amount sufficient to inhibit proliferation of such cells and preferably in an amount sufficient to lead to cancer cell death, as an active ingredient. Of course, the composition can be formulated so as to provide slow or controlled release of the active ingredient using known pharmacological procedures, such as, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile.

Topical or transdermal administration may be by way of applying powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants that may be required. Ophthalmic formulations in the form of eye ointments, powders, solutions and the like, may also be used in the method of the invention.

The method of the invention contemplates the administration of the agent which causes increased intracellular granularity in cancer cells as the active ingredient, as pharmaceuticals to humans and animals, administered per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of the active ingredient in combination with a pharmaceutically acceptable carrier. Actual dosage levels of the active ingredient may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response from an individual patient, and given the choice of agent, mode of administration, etc.

The actual dosage depends upon a variety of factors including the activity of the particular compound of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like
factors well known in the medical arts, and it is within the ordinary skill of a physician to
determine the effective dose.

In general, a suitable daily dose of a compound of the invention will be that amount of
the compound that is the lowest dose effective to produce a therapeutic effect. Such an
effective dose will generally depend upon the factors described above. Generally, doses will
range from about 0.0001 to about 100 mg per kilogram of body weight per day.

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

"Treat", "treating", and "treatment", etc., as used herein, refer to any action providing
a benefit to a patient at risk for or afflicted by cancer, including improvement in the condition
through lessening or suppression of at least one symptom, delay in progression of the effects
of the cancer, prevention or delay in the onset of the effects of the cancer, etc. Treatment, as
used herein, encompasses both prophylactic and therapeutic treatment.

As discussed above, for example, the agents which cause increased intracellular
granularity in cancer cells, at least in an amount sufficient to inhibit proliferation of such cells
and preferably in an amount sufficient to lead to cancer cell death, and so can be administered
prophylactically to a mammal in advance of the initiation or proliferation of such cancer.
Prophylactic administration is effective to decrease the likelihood of the occurrence of
disease in the mammal.

It will be apparent to those skilled in the art that various modifications and variations
can be made in the devices and methods of the present disclosure. Other embodiments of the
disclosure will be apparent to those skilled in the art from consideration of the specification
and practice of the embodiments disclosed herein. It is intended that the specification and
examples be considered as exemplary only.

The complete disclosure of all patents, patent applications, and publications, and
electronically available material cited herein are incorporated by reference. Any
inconsistency between the material incorporated by reference and the material set for in the
specification as originally filed shall be resolved in favor of the specification as originally
filed. The foregoing detailed description and examples have been given for clarity of
understanding only. No unnecessary limitations are to be understood therefrom. The
invention is not limited to the exact details shown and described, for variations obvious to
one skilled in the art will be included within the invention defined by the claims.
All headings are for the convenience of the reader and should not be used to limit the meaning of the text that follows the heading, unless so specified.
CLAIMS:

1. A method of reducing the likelihood of cancer in a mammal comprising administering to said mammal an agent which causes increased intracellular granularity in cancer cells in an amount sufficient to cause cancer cell death.

2. A method of treating refractory cancer in a patient, particularly hormone refractory prostate cancer, comprising administering to said patient an agent which causes increased intracellular granularity in the refractory cancer cells, at least in an amount sufficient to inhibit proliferation of such cells and preferably in an amount sufficient to lead to refractory cancer cell death.

3. A method of treating cancer in a patent in need of said treatment comprising administering to the patient an effective amount of an agent which causes an increase in intracellular granularity of cancer cells leading to cancer cell death.

4. A method of treating cancer in a patient in need of said treatment comprising administering an agent to said patient in an amount effective for converting adherent cancer cells to non-adherent cancer cells, leading to cancer cell death.


6. The method of claims 1-5 wherein said agent is selected from the group consisting of 3-hydroxymethyl-beta-carboline, Albendazole, Alprenolol hydrochloride, Ambroxol, hydrochloride, Amitryptiline hydrochloride, Amodiaquin dihydrochloride dihydrate, Amoxapine, Antazoline hydrochloride, Anisomycin, Antimycin A, Apomorphine hydrochloride, Astemizole, Azacyclonol, Benperidol, Benzethonium chloride, Benzydamine hydrochloride, Bepridil hydrochloride, Bromperidol, Cephaeline, dihydrochloride
heptahydrate, Chelidonine monohydrate, Chloropyramine hydrochloride, Chlorotrianisene, Chlorpromazine hydrochloride, Chlo\textcircled{o} rothixene hydrochloride, Chrysene-1, 4-quinone, Clomiphene citrate, Clomipramine hydrochloride, Corticosterone, Cyclobenzaprine hydrochloride, Cyproheptadine hydrochloride, Diaziquone, Dibucaine, Dienestrol, Digitoxigenin, Digoxin, Dihydroergocristine mesylate, Dihydroergotoxine mesylate, Domperidone, Doxepin hydrochloride, Emetine dihydrochloride, Epiandrosterone, Ethisterone, Fenbendazole, Fendiline hydrochloride, Fluoxetine hydrochloride, Fluphenazine dihydrochloride, GBR 12909 dihydrochloride, Gossypol, Gramicidin, 7-benzyloxy Gramine, Haloprogin, Harmaline hydrochloride dehydrate, Hycanthone, Ifenprodil tartrate, Imipramine hydrochloride, Ketonifen fumarate, Lanatoside C, Lidoflazine, Loperamide hydrochloride, Maprotiline hydrochloride, Mebendazole, Methimazole, Methotrimeprazine maleate, Methiothepin maleate, Methotrexate maleate, Methoxyflurane, Mycophenolic acid, Niclosamide, Nitrarine dihydrochloride, Nocodazole, Norcyclobenzaprine, Norethynodrel, Nortriptyline hydrochloride, Parthenolide, Pe\textcircled{o} henazine, Pemexiline maleate, Pimozide, Piperlongumine, Phenofbrmin hydrochloride, Primaquine diphosphate, Prochlorperazine dimaleate, Propafenone hydrochloride, Pyrimethamine, Quinacrine dihydrochloride dihydrate, Quinidine hydrochloride monohydrate, Remerine hydrochloride, Rescinnamin, Securinine, Secourine, Solanine alpha, Strophanthidin, Sulocitidil, Tacrine hydrochloride hydrate, Terconazole, Terfenadine, Thimerosal, Thioridazine hydrochloride, 1-phenyl-3-(2-thiazolyl)-2-Thiourea, Triamterene, Trifluoperazine dihydrochloride, Triflupromazine hydrochloride, Trimeprazine tartrate, Trimipramine maleate salt, Ursolic acid, Zimelidine dihydrochloride monohydrate, a pharmaceutically acceptable salt or alternative pharmaceutically acceptable salt and combinations thereof.

7. The method of claims 1-6 wherein the agent is astemizole.

8. The method of claim 6 or 7 wherein the cancer is prostate cancer.

9. The method of claims 1-8 wherein said agent is administered as a pharmaceutical composition comprising a pharmaceutically acceptable carrier and the agent.
10. The method according to any of claims 1-9 wherein said agent is coadministered with an effective amount of at least one secondary anticancer agent.

11. The method according to claim 10 wherein said secondary anticancer agent is an antimetabolite, an inhibitor of topoisomerase I and II, an alkylating agent, a microtubule inhibitor or mixtures thereof.

12. The method according to claim 10 wherein said secondary anticancer compound is selected from the group consisting of aldesleukin; aemtuzumab; altiretinoin; allopurinol; altretamine; amifostine; anastrozole; arsenic trioxide; aparaginase; BCG Live; bexarotene capsules; bexarotene gel; bleomycin; busulfan intravenous; busulfan oral; calusterone; capcitabine; carboplatin; carmustine; carmustine with poiseprosan 20 implant; celecoxib; chlorambucil; cisplatin; cladribine; cyclophosphamide; cytarabine; cytarabine liposomal; dacarbazine; dactinomycin; actinomycin D; dabepoetin alfa; daunorubicin liposomal; daunorubicin; daunomycin; dinoileukin diftitox; dextrazoxane; docetaxel; doxorubicin; doxorubicin liposomal; domostanolone propionate; eliott's B soution; epirubicin; etoetin alfa estramustine; etoposide phosphate; etoposide (VP-16); exemestane; flgrastim; flouxuridine (intraarterial); fludarabine; fluorouracil (5-FU); fulvestrant; gemtuzumab ozogamicin; goserebin acetate; hydroxyurea; Ibritumomab Tiuxetan; idarubicin; ifosfamide; imatinib mesylate; Interferon alfa-2a; Interferon alfa-2b; irinotecan; letrozole; leucovorin; levamisole; lomustine (CCNU); mecloethamine (nitrogen mustard); megestrol acetate; melphalan (L-PAM); mercaptopurine (6-MP); mesna; methotrexate; methoxsalen; mitomycin C; mitotane; mitoxantrone; nandrolone phenpropionate; n fetumomab; LOddC; orelvekin; oxaliplatin; paclitaxel; pamidronate; pegademase; Pegaspargase; Pegfilgfastirh; pefostatirh; pipobromnariT- plicamycin; mithramycin; porfimer sodium; procarbazine; quinacrine; Rasburicase; Rituximab; Sargamostim; streptozocin; talbudidine (LDT); tale; tamoxifen; temozolomide; teniposide (VM-26); testolactone; thioguanine (6-TG); thiopeta; topotecan; toremifene; Tositumomab; Trastuzumab; tretinoin (ATRA); Uracil Mustard; valrubicin; valtorcitabine (monoval LDC); vinblastine; vinorelbine; zoledronate; a pharmaceutically acceptable salt thereof and mixtures thereof.

13. The method according to claim 8 wherein said agent is coadministered with at least one antiandrogen compound.
14. The method according to claim 8 or 13 wherein said agent is coadministered with at least one GnRH modulator.

15. The method according to claim 8, 13 or 14 wherein said agent is coadministered with at least one medicament selected from the group consisting of flutamide, bicalutamide, nilutamide, cyproterone acetate, ketoconazole, aminoglutethimide, abarelix, leuprolide, goserelin, triptorelin, buserelin, abiraterone acetate, sorafenib and mixtures thereof.

16. The method according to claim 8, 13 or 14 wherein said agent is coadministered with at least one medicament selected from the group consisting of an enlarged prostate agent, eulexin, flutamide, goserelin, leuprolide, lupron, nilandron, nilutamide, zoladex and mixtures thereof.

17. The method according to any of claims 1-17 used in combination with radiation therapy.

18. A pharmaceutical composition comprising an anti-cancer effective amount of a compound selected from the group consisting of 3-hydroxymethyl-beta-carboline, Albendazole, Alprenolol hydrochloride, Ambroxol, hydrochloride, Amitryptiline hydrochloride, Amodiaquin dihydrochloride dihydrate, Amoxapine, Antazoline hydrochloride, Anisomycin, Antimycin A, Apomorphine hydrochloride, Astemizole, Azacyclonol, Benperidol, Benzethonium chloride, Benzydamine hydrochloride, Bepridil hydrochloride, Bromperidol, Cephaeline, dihydrochloride heptahydrate, Chelidonine monohydrate, Chlorpyramine hydrochloride, Chlorotrianisene, Chlorpromazine hydrochloride, Chlorprothixene hydrochloride, Chrysene-1, 4-quinone, Clomiphene citrate, Clomipramine hydrochloride, Corticosterone, Cyclobenzaprine hydrochloride, Cyproheptadine hydrochloride, Diaziquone, Dibucaine, Dienestrol, Digitoxigenin, Digoxin, Dihydroergocristine mesylate, Dihydroergotoxine mesylate, Domperidone, Doxepin hydrochloride, Emetine dihydrochloride, Epiandrosterone, Ethisterone, Fenbendazole, Fendiline hydrochloride, Fluoxetine hydrochloride, Fluphenazine dihydrochloride, GBR 12909 dihydrochloride, Gossypol, Granicidin, 7-benzyloxy Gramine, Haloprogyn, Harmaline hydrochloride dehydrate, Hycanthone, Ifenprodil tartrate, Imipramine hydrochloride, Ketotifen fumarate, Lanatoside C, Lidoflazine, Loperamide hydrochloride, Maprotiline hydrochloride, Mebendazole, Mebhydroline 1,5-naphtalenesulfonate, Menadione,
Mesoridazine besylate, Metergoline, Methimazole, Methiothepin maleate, Methotrimeprazine maleate salt, Metixene hydrochloride, Mycophenolic acid, Niclosamide, Nitrarime dihydrochloride, Nocodazole, Norcyclobenzaprine, Norethynodrel, Nortriptyline hydrochloride, Parthenolide, PeΦ henazine, Pemexiline maleate, Pimozide, Piperlongumine, Phenoformin hydrochloride, Primaquine diphosphate, Prochlorperazine dimaleate, Propafenone hydrochloride, Pyrimethamine, Quinacrine dihydrochloride dihydrate, Quinidine hydrochloride monohydrate, Remerine hydrochloride, Rescinnamin, Securinine, Scoulerine, Solanine, Strophanthidin, Sulcotidil, Tacrine hydrochloride hydrate, Terconazole, Terfenadine, Thimerosal, Thioridazine hydrochloride, l-phenyl-3-(2-thiazolyl)-2-Thiourea, Triamterene, Trifluoperazine dihydrochloride, Triflupromazine hydrochloride, Trimeprazine tartrate, Trimipramine maleate salt, Ursolic acid, Zimelidine dihydrochloride monohydrate, a pharmaceutically acceptable salt or an alternative pharmaceutically acceptable salt and combinations thereof in combination with at least one secondary anticancer agent and a pharmaceutically acceptable carrier, additive or excipient.

19. The composition according to claim 18 wherein said secondary anticancer agent is an antimetabolite, an inhibitor of topoisomerase I and II, an alkylating agent, a microtubule inhibitor or mixtures thereof.

20. The composition according to claim 18 wherein said secondary anticancer agent is selected from the group consisting of aemtuzumab; alitretinoin; allopurinol; altretamine; amifostine; anastrozole; arsenic trioxide; aparaginase; BCG Live; bexarotene capsules; bexarotene gel; bleomycin; busulfan intravenous; busulfan oral; calusterone; capecitabine; carboplatin; carmustine; carmustine with poifepropan 20 implant; celecoxib; chlorambucil; cisplatin; cladribine; cyclophosphamide; cytarabine; cytarabine liposomal; dacarbazine; daunorubicin; daunomycin; dactinomycin; dactinomycin D; dabapoetin alfa; daunorubicin liposomal; daunorubicin, daunomycin; dnileukin histidixo, dexrazoxane; docetaxel; doxorubicin; doxorubicin liposomal; domostanalone propionate; eliott's B solution; epirubicin; etoetin alfa; estramustine; etoposide phosphate; etoposide (VP-16); exemestane; flgrastim; floxuridine (intraarterial); fludarabine; fluorouracil (5-FU); fulvestrant; gemtuzumab ozogamicin; gosserelin acetate; hydroxyurea; Ibritumomab Tiuxetan; idarubicin; ifosfamide; imatinib mesylate; Interferon alfa-2a; Interferon alfa-2b; irinotecan; letrozole; leucovorin; levamisole; lomustine (CCNU); meclozuramine (nitrogen mustard); megestrol acetate; melphalan (L-PAM); mercaptopurine (6-MP); mesna; methotrexate; methoxsalen; mitomycin C; mitotane;
mitoxantrone; nandrolone phenpropionate; nfetumomab; OD; orelvekin; oxaliplatin; paclitaxel; pamidronate; pegademase; Pegasparagase; Pegfilgrastim; pentostatin; pipobroman; plicamycin; mithramycin; porfimer sodium; procarbazine; quinacrine; Rasburicase; Rituximab; Sargramostim; streptozocin; talbuvidine (LDT); talc; tamoxifen; temozolomide; teniposide (VM-26); testolactone; thioguanine (6-TG); thiopeta; topotecan; toremifene; Tositumomab; Trastuzumab; tretinoin (ATRA); Uracil Mustard; valrucin; valtorcitabine (monoval LDC); vinblastine; vinorelbine; zoledronate.

21. A pharmaceutical composition comprising an anti-cancer effective amount of a compound selected from the group consisting of 3-hydroxymethyl-beta-carboline, Albendazole, Alprenolol hydrochloride, Ambroxol, hydrochloride, Amitryptiline hydrochloride, Amodiaquin dihydrochloride dihydrate, Amoxapine, Antazoline hydrochloride, Anisomycin, Antimycin A, Apomorphine hydrochloride, Astemizole, Azacyclonol, Benperidol, Benzedrinum chloride, Benzodamine hydrochloride, Bepridil hydrochloride, Bromperidol, Cephaeline, dihydrochloride heptahydrate, Chelidonine monohydrate, Chlorpropane hydrochloride, Chlorotrianisene, Chlo® romazine hydrochloride, Chlorprothixene hydrochloride, Chrysene-1,4-quinone, Clomiphene citrate, Clomipramine hydrochloride, Corticosterone, Cyclobenzaprine hydrochloride, Cyproheptadine hydrochloride, Diaziquone, Dibucaine, Diencol, Digitoxigenin, Digoxin, Dihydroergocristine mesylate, Dihydroergotoxine mesylate, Domperidone, Doxepin hydrochloride, Emetine dihydrochloride, Epiandrosterone, Ethisterone, Fenbendazole, Fendiline hydrochloride, Fluoxetine hydrochloride, Fluphenazine dihydrochloride, GBR 12909 dihydrochloride, Gossypol, Gramicidin, 7-benzyloxy Gramine, Haloprogin, Harmaline hydrochloride dehydrate, Hycanthone, Ifenprodil tartrate, Imipramine hydrochloride, Ketotifen fumarate, Lanatoside C, Lidoflazine, Loperamide hydrochloride, Maprotiline hydrochloride, Mebendazole, Mebhydroline 1,5-naphtalenesulfonate, Menadione, Mesoridazine besylate, Metergoline, Methimazole, Methiothepin maleate, Methotrinepazine maleate salt, Metixene hydrochloride, Mycophenolic acid, Niclosamide, Nitrarine dihydrochloride, Nocodazole, Norcyclobenzaprine, Norethynodrel, Nortriptyline hydrochloride, Parthenolide, Perphenazine, Penemxiline maleate, Pimozide, Piperlongumine, Phenoformin hydrochloride, Primaquine diphosphate, Prochlorperazine dimaleate, Propafenone hydrochloride, Pyrimethamine, Quinacrine dihydrochloride dihydrate, Quinidine hydrochloride monohydrate, Remerine hydrochloride, Rescinnamin, Securinine, Scoulerine, Solanine alpha, Strophanthidin, Sulocitidil, Tacrine hydrochloride
hydrate, Terconazole, Terfenadine, Thimerosal, Thioridazine hydrochloride, 1-phenyl-3-(2-thiazolyl)-2-Thiourea, Triamterene, Trifluoperazine dihydrochloride, Triflupromazine hydrochloride, Trimeprazine tartrate, Trimipramine maleate salt, Ursolic acid, Zimelidine dihydrochloride monohydrate, a pharmaceutically acceptable salt or alternative pharmaceutically acceptable salt and combinations thereof further in combination with at least one secondary agent useful for treating prostate cancer, in further combination with a pharmaceutically acceptable carrier, additive or excipient.

22. The composition according to claim 21 wherein said secondary agent is at least one antiandrogen compound.

23. The composition according to claim 21 wherein said secondary agent is at least one GNRH modulator.

24. The composition according to claim 21 wherein said secondary agent is at least one medicament selected from the group consisting of flutamide, bicalutamide, nilutamide, cyproterone acetate, ketoconazole, aminoglutethimide, abarelix, leuprolide, goserelin, triptorelin, buserelin, abiraterone acetate, sorafenib and mixtures thereof.

25. The composition according to claim 21 wherein said secondary agent is at least one medicament selected from the group consisting of an enlarged prostate agent, eulexin, flutamide, goserelin, leuprolide, lupron, nilandron, nilutamide, zoladex and mixtures thereof.

26. Use of an agent in the manufacture of a medicament for the treatment of cancer, said agent being selected from the group consisting of 3-hydroxymethyl-beta-carboline, Albendazole, Alprenolol hydrochloride, Ambroxol, hydrochloride^Amitryptiline - hydrochloride, Amodiaquin dihydrochloride dihydrate, Amoxapine, Antazoline hydrochloride, Anisomycin, Antimycin A, Apomorphine hydrochloride, Astemizole, Azacyclonol, Benperidol, Benzethonium chloride, Benzydamine hydrochloride, Bepridil hydrochloride, Bromperidol, Cephaeline, dihydrochloride heptahydrate, Chelidonine monohydrate, Chlorpropyramine hydrochloride, Chlorotrianisene, Chlorpromazine hydrochloride, Chlorprothixene hydrochloride, Chrysene-1, 4-quinone, Clomiphene citrate, Clomipramine hydrochloride, Corticosterone, Cyclobenzaprine hydrochloride, Cyproheptadine hydrochloride, Diaziquone, Dibucaine, Dienestrol, Digitoxigenin, Digoxin,
Dihydroergocristine mesylate, Dihydroergotoxine mesylate, Domperidone, Doxepin hydrochloride, Emetine dihydrochloride, Epiandrosterone, Ethisterone, Fenbendazole, Fendiline hydrochloride, Fluoxetine hydrochloride, Fluphenazine dihydrochloride, GBR 12909 dihydrochloride, Gossypol, Gramicidin, 7-benzyloxy Gramine, Haloprogin, Harmaline hydrochloride dehydrate, Hycanthone, Ifenprodil tartrate, Imipramine hydrochloride, Ketotifen fumarate, Lanatoside C, Lidoflazine, Loperamide hydrochloride, Maprotiline hydrochloride, Mebendazole, Mebhydroline 1,5-naphtalenesulfonate, Menadione, Mesoridazine besylate, Metergoline, Methimazole, Methiothepin maleate, Methotrimeprazine maleate salt, Metixene hydrochloride, Mycophenolic acid, Niclosamide, Nitrarine dihydrochloride, Nocodazole, Norcyclobenzaprine, Norethynodrel, Nortriptyline hydrochloride, Parthenolide, Peφhenazine, Pemexiline maleate, Pimozide, Piperlongumine, Phenoformin hydrochloride, Primaquine diphosphate, Prochlorperazine dimaleate, Propafenone hydrochloride, Pyrimethamine, Quinacrine dihydrochloride dihydrate, Quinidine hydrochloride monohydrate, Remerine hydrochloride, Rescinnamin, Securinine, Scoulerine, Solanine alpha, Strophanthinidin, Suloctidil, Tacrine hydrochloride hydrate, Terconazole, Terfenadine, Thimerosal, Thioridazine hydrochloride, 1-phenyl-3-(2-thiazolyl)-2-Thiourea, Triamterene, Trifluperazine dihydrochloride, Triflupromazine hydrochloride, Trimeprazine tartrate, Trimipramine maleate salt, Ursolic acid, Zimelidine dihydrochloride monohydrate, a pharmaceutically acceptable salt or an alternative pharmaceutically acceptable salt and combinations thereof.

27. Use according to claim 26 wherein said agent is astemizole.

28. Use according to claim 26 or 27 wherein said agent is combined with a secondary agent.

29. Use according to claim 29 wherein said secondary agent is a secondary anticancer agent selected from the group consisting of an antimetabolite, an inhibitor of topoisomerase I and II, an alkylating agent, a microtubule inhibitor or mixtures thereof.

30. Use according to 26 or 27 wherein said agent is combined with a secondary anticancer agent selected from the group consisting of aldesleukin; aemtuzumab; alitretinoin; allopurinol; altretamine; amifostine; anastrozole; arsenic trioxide; aparaginase; BCG Live; bexarotene capsules; bexarotene gel; bleomycin; busulfan intravenous; busulfan oral;
calusterone; capecitabine; carboplatin; carmustine; carmustine with poifeprosan 20 iplant; celecoxib; chlorambucil; cisplatin; cladribine; cyclophosphamide; cytarabine; cytarabine liposomal; dacarbazine; dactinomycin; actinomycin D; dabepoetin alfa; daunorubicin liposomal; daunorubicin, daunomycin; dnileukin dittox, dexamoxan; docetaxel; doxorubicin liposomal; daunorubicin, daunomycin, melphalan (L-PAM); mercaptopurine (6-MP); mesna; methotrexate; methoxsalen; mitomycin C; mitotane; mitoxantrone; nandrolone phenpropionate; nfetumomab; LOddC; orelvekin; oxaliplatin; paclitaxel; pamidronate; pegademase; Pegaspargase; Pegfilgrastim; pentostatin; pipobroman; plicamycin; mithramycin; porfimer sodium; procarbazine; quinacrine; Rasburicase; Rituximab; Sargramostim; streptozocin; talbuvidine (LDT); talc; temoxifen; temozolomide; teniposide (VM-26); testolactone; thioguanine (6-TG); thiopeta; topotecan; toremifene; Tositumomab; Trastuzumab; tretinoin (ATRA); Uracil Mustard; valrubcin; valtorcitabine (monoval LDC); vinblastine; vinorelbine; zoledronate.

31. Use according to any of claims 26-30 wherein said agent(s) is combined with radiation therapy.

32. Use according to claim 28 wherein said secondary agent is at least one antiandrogen compound.

33. Use according to claim 28 wherein said secondary agent is at least one GnRH modulator.

34. Use according to claim 28 wherein said secondary agent is at least one medicament selected from the group consisting of flutamide, bicalutamide, nilutamide, cyproterone acetate, ketoconazole, aminoglutethimide, abarelix, leuprolide, goserelin, triptorelin, buserelin, abiraterone acetate, sorafenib and mixtures thereof.
35. Use according to claim 28 wherein said secondary agent is at least one medicament selected from the group consisting of an enlarged prostate agent, eulexin, flutamide, goserelin, leuprolide, lupron, nilandron, nilutamide, zoladex and mixtures thereof.
Fig. 1

OCH₃

Fig. 2

2A. Control

2B. Treated

SUBSTITUTE SHEET (RULE 26)
3A. Control

3B. Treated

Fig. 3

Fig. 4
Fig. 5

Prostate cancer cells

Skin cancer cells

Fig. 6

Astromizole Viability Dose-Response in PC3 Cells at 48hr
Fig. 7

DU145

PC3

LAPC4

LNCAP

Cell Growth (Relative Fluorescence Units)

Cell Growth (Relative Fluorescence Units)

Cell Growth (Relative Fluorescence Units)

Cell Growth (Relative Fluorescence Units)

0 1 2 3 4 5 6 7 8 9 10

[Astemizole] (uM)

[Astemizole] (uM)

[Astemizole] (uM)

[Astemizole] (uM)

7A

7B

7C

7D

Fig. 8

Untreated

10 uM Ast

LC3 I

LC3 II

actin