(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 December 2008 (18.12.2008)

PCT

(10) International Publication Number
WO 2008/154382 A1

(51) International Patent Classification:
A61K 31/495 (2006.01) *C07C 259/10* (2006.01)
A61K 31/44 (2006.01) *C07D 215/20* (2006.01)
C07D 233/54 (2006.01)

(21) International Application Number:
PCT/US2008/066120

(22) International Filing Date: 6 June 2008 (06.06.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/942,452 6 June 2007 (06.06.2007) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

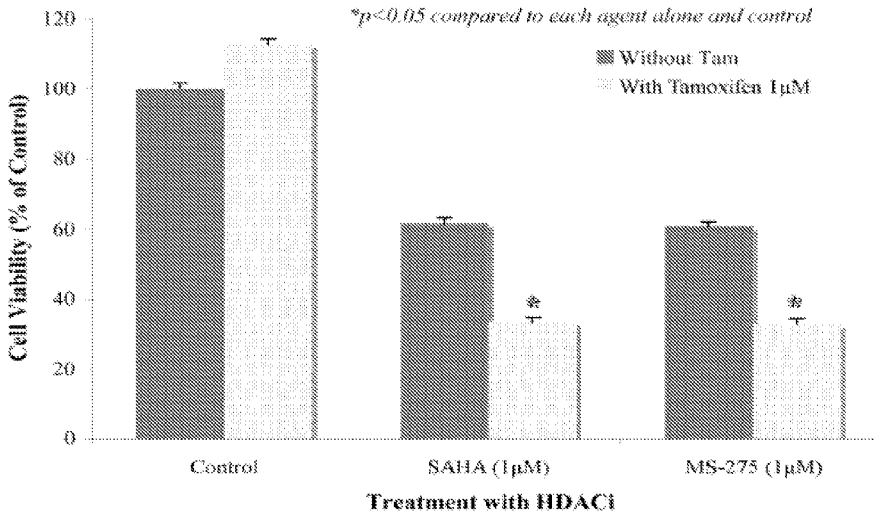
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

[Continued on next page]

(54) **Title:** HDAC INHIBITORS AND HORMONE TARGETED DRUGS FOR THE TREATMENT OF CANCER

FIG. 1A



(57) **Abstract:** The present invention relates to the methods of treating endocrine-regulated cancers, including hormone resistant cancers, for example. More specifically, the present invention relates to a method of increasing the sensitivity of hormone resistant cancers to hormonal therapeutic agents. In particular aspects, the present invention concerns delivery of a histone deacetylase inhibitor and a hormone targeted drug to an individual with cancer. In further particular aspects, the histone deacetylase inhibitor and the hormone targeted drug act synergistically to treat the cancer, including by overcoming resistance to a cancer therapy.

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— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

HDAC INHIBITORS AND HORMONE TARGETED DRUGS FOR THE TREATMENT OF CANCER

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 60/942,452, filed June 6, 2007, and to U.S. Provisional Patent Application Serial No. 61/013,570, filed December 13, 2007, both of which applications are incorporated by reference herein in their entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made in part with government support under Grant Nos. R01 CA-62483 and R21 CA117991 awarded by the National Institutes of Health. The United States Government has certain rights in the invention.

TECHNICAL FIELD

[0003] The present invention relates at least to the fields of medicine and oncology. More specifically, the present invention relates to the methods of treating cancers, including hormone-resistant cancers, for example. In addition to treating the exemplary hormone-resistant cancers, the present invention can be used to increase the sensitivity of cancers to therapy, such as increasing the sensitivity of hormone-resistant cancers to hormonal therapeutic agents, by administering a combination of one or more histone deacetylase inhibitors and one or more hormonal therapeutic agents.

BACKGROUND OF THE INVENTION

[0004] Hormone resistance is a particular problem in cancers such as prostate cancer and breast cancer. Androgens, acting via androgen receptors, are essential for normal growth and function of the prostate gland and have been implicated in the progression of prostate cancer. Selective androgen receptor modulators (SARMs) --drugs intended to inhibit the activity of androgen receptors--are therefore standard treatment for prostate cancer. However, prostate cancers often become resistant to such treatment. A similar phenomenon can also occur in breast cancers treated with drugs that target the hormone receptor for estrogen.

A. Breast Cancer

[0005] Breast cancer is the most prevalent form of cancer among women in the United States and second leading cause of cancer related deaths (Jemal et al., 2006). According to 2006 cancer statistics, approximately 40,000 women are expected to die from breast cancer in the US (Jemal et al. 2006). Although, in the year 2003, a marked 7% decrease in the incidence of breast cancer was reported, this decrease mainly was associated with ER+ breast cancers (Ravdin et al. 2006). ER- breast cancer still is essentially incurable and aggressive. Although, breast cancer treatment has undergone significant improvement, cancer develops resistance to almost all forms of therapy. Additionally, there has been no improvement in the treatment of ER- breast cancer. The high prevalence of breast cancer and development of resistance to effective treatments provides a strong stimulus for the development of additional, targeted therapies with minimum toxicity.

[0006] The knowledge that steroids contribute a pivotal role in development of breast cancer has been exploited clinically by the development of endocrine agents, predominantly by estrogen withdrawal or antagonism (Jordan et al. 2007). Antiestrogen tamoxifen has been used to treat breast cancer for several years now. More recently, AIs such as letrozole and anastrozole have surpassed the beneficiary effects of tamoxifen and are now being used in the clinic as the first line treatment for hormone dependent post-menopausal breast cancer (Goss et al. 2005; Goss et al. 2002). AEs/ AIs are currently used for postmenopausal ER positive breast cancer agents (Brodie, 1990; Baum, 2002; Baum et al., 2002). The clinical use of these agents is hampered by development of resistance and the presence of ER- cancer phenotype. Loss of AE/AI sensitivity has been associated with lack of ER expression.

B. Prostate Cancer

[0007] Worldwide, 543,000 new cases of prostate cancer are currently diagnosed each year (Jemal et al. Cancer statistics, 2003. CA Cancer J Clin 2003; 53: 5–26). In the USA, it is the most commonly diagnosed cancer in men, with an incidence of 104 cases per 100,000. The estimated lifetime risk of getting prostate cancer is 30% and the lifetime risk of dying from the disease is about 3% (Jemal et al. Cancer statistics, 2003. CA Cancer J Clin 2003; 53: 5–26). Prostate cancer is the most common malignancy, and second leading cause of cancer related deaths in men in the western world. The incidence and mortality rates from prostate cancer are increasing and this is due, in part, to an increasingly aging population and the higher incidence of this disease in older men (Gao et al., 1997; Chiarodo, 1991). Both benign prostatic hypertrophy (BPH) and prostate cancer are decreased or not detected in eunuchs and are linked not only to

advancing age but the presence of testes and androgen function (Gao et al., 1997; Chiarodo, 1991; Sakti and Crawford, 1993).

[0008] The identification of prostate specific antigen (PSA), an androgen receptor (AR) target gene, as a biomarker for the disease has helped improve early diagnosis leading to significant improvements in treatment. Early detection is vital for PCa therapy as localized disease can be effectively treated—usually with surgery and/or radiotherapy. Unfortunately, approximately 9% of patients present advanced disease upon diagnosis and 18% of patients treated for localized PCa develop metastatic disease (ACS, Cancer Facts and Figures 2007. 2007, American Cancer Society: Atlanta; Pound et al. JAMA, 1999. 281(17): p. 1591-7). Androgen ablation and chemotherapy remain the standard treatments for advanced PCa, but unfortunately these strategies are limited and the prognosis for patients with advanced disease is bleak.

[0009] Androgens play a vital role in the development, growth, and progression of PCa (McConnell, J.D., Urol Clin North Am, 1991. 18(1): p. 1-13). At the molecular level, androgens, mainly testosterone (T) and dihydrotestosterone (DHT), bind to the AR and initiate transcription of genes involved in cell proliferation and survival (Edwards et al. BJU Int, 2005. 95(9): p. 1320-6; Klaassen et al. Br J Cancer, 2001. 85(4): p. 630-5). The testes synthesize about 90% of T, with the remaining 10% produced by the adrenal glands. T can be subsequently converted DHT by the enzyme steroid 5α-reductase that is localized primarily in the prostate (Bruchovsky et al. J Biol Chem, 1968. 243(8): p. 2012-21).

[0010] The benefits of androgen deprivation therapy for PCa treatment were first demonstrated by Charles Huggins and colleagues in 1941 (Huggins et al. Cancer Res, 1941. 1: p. 293-297; Huggins et al. Arch Surgery, 1941. 43: p. 209). It is estimated that 80-90% of patients show initial response to androgen deprivation therapy (ADT) (Denis et al. Cancer, 1993. 72(12 Suppl): p. 3888-95). Generally, ADT is carried out via chemical castration with LHRH or GnRH agonists. AR antagonists such as bicalutamide and flutamide are also used either alone or in conjunction with castration to prevent binding of adrenal androgens to the AR.

[0011] Early prostate cancer tends to be androgen-dependent and requires expression of a functional androgen receptor (AR), whereas later stage tumors progress to androgen-independence which in some cases is correlated with loss of AR function (Cheng et al., 1993). Interestingly, the progression from early stage hormone-dependent to latter stage

hormone-independence in prostate cancer in men is also observed for breast cancer in women where estrogen-responsiveness undergoes a similar pattern of change in women with early or late stage disease (Hopp and Fuqua, 1999; Fuqua et al., 1995).

[0012] Prostate cancer therapy is dependent on the stage of the tumor and AR expression. Early stage androgen-responsive prostate cancers can be treated by castration or with antiandrogens or drugs that block androgen-induced responses including steroidal antiandrogens (cyproterone), LHRH analogs, nonsteroidal antiandrogens (flutamide, nilutamide, bicalutamide), and the potent estrogenic drug diethylstilbestrol (reviewed in (Sadar et al., 1999; Klotz, 2000; Morris et al., 2000; Boccardo, 2000). In addition, there are several possible novel strategies for treatment of prostate cancer and other tumor-types and these include targeting of critical genes involved in tumor cell growth and metastasis (e.g., antiangiogenic drugs, antisense therapy) (Boasberg et al., 1997; Knox et al.,1998; 1998; Yamaoka et al.,1993; Folkman, 1995; Folkman, 1971). Ligands for nuclear receptors (NR) are also being developed for treatment of prostate cancer through inhibitory NR-AR crosstalk that involves various ligands or drugs that bind the retinoid acid/X-receptors (retinoids), vitamin D receptor (calcitrol), and peroxisome proliferator activate receptor γ (trogilatazone) (Dorai et al., 1997; Pienta et al., 1993; Pollard et al., 1991; Kelly et al., 1996; Miller et al., 1992; Miller et al., 1995; Peehl et al., 1994; Gross et al., 1998; Kubota et al., 1998; Tontonoz et al., 1997; Tontonoz et al., 1994; Smith et al., 1999).

[0013] The present invention is the first to use a combination of a targeted hormone agent and histone deacetylase inhibitors to treat hormone resistant cancers.

SUMMARY OF THE INVENTION

[0014] In particular aspects, the present invention concerns treating endocrine-regulated cancers, including, by way of non-limiting example, prostate, testicular, seminal vesicle, breast, uterine, ovary, and cervical cancer. In other particular aspects, the present invention concerns treating benign prostatic hyperplasia. In further particular aspects, the present invention concerns diagnosing prostate cancer or benign prostatic hyperplasia. In specific aspects, the endocrine-regulated cancer is resistant to one or more therapies, whereas in other aspects the cancer is sensitive to one or more therapies. In further specific aspects, the endocrine-regulated cancer is resistant to one or more hormone therapies, whereas in other

aspects the cancer is sensitive to one or more hormone therapies. In particular aspects, the cancer is hormone-resistant cancer, such as, for example, androgen resistant cancer.

[0015] In certain embodiments, the invention is drawn to a method of treating a hyperplasia disease, which was originally hormone dependent but has become refractory to hormone therapy, comprising administering to a subject having or suspected of having the hyperplasia disease a histone deacetylase inhibitor and a compound which is both an androgen receptor antagonist and a 17alpha-hydroxylase-C17,20-lyase inhibitor, in an effective amount to treat the hyperplasia disease.

[0016] In specific embodiments, the hyperplasia disease is a refractory cancer. In particular embodiments, the hyperplasia disease is selected from the group consisting of prostate cancer, testicular cancer and cancer of the seminal vesicles. In further particular embodiments, the hyperplasia disease is prostate cancer.

[0017] In other specific embodiments, the hyperplasia disease is selected from the group consisting of breast cancer, uterine cancer, ovarian cancer, and cervical cancer.

[0018] In further other specific embodiments, the hyperplasia disease is benign prostatic hyperplasia.

[0019] In specific embodiments, the histone deacetylase inhibitor is selected from the group consisting of suberoylanilide hydroxamic acid (SAHA), CI-994, MS-275, 3-(1-Methyl-4-phenylacetyl-1H-2-pyrrolyl)-N-hydroxy-2-propenamide (APHA), apicidin, sodium butyrate, (-)-depudecin, scriptaid, sirtinol, trichostatin A, romidepsin, belinostat, LAQ-824, LBH-589, MGCD-0103, KD-5170, depsipeptide, and any combination thereof.

[0020] In further embodiments, the histone deacetylase inhibitor is SAHA. In other further embodiments, the histone deacetylase inhibitor is romidepsin.

[0021] In specific embodiments, the compound which is both an androgen receptor antagonist and a 17-alpha-hydroxylase-C17,20-lyase inhibitor is 3beta-hydroxy-17-(1H-benzimidazole-1-yl)androsta-5,16-diene.

[0022] In certain embodiments, methods of the invention further comprise administering an effective amount of an androgen receptor antagonist selected from the group

consisting of bicalutamide, flutamide, cyproterone acetate and/or a hormone therapy selected from ketoconazole or abiraterone.

[0023] In other certain embodiments, the histone deacetylase inhibitor and the compound which is both an androgen receptor antagonist and a 17-alpha-hydroxylase-C17,20-lyase inhibitor are administered simultaneously. In further other certain embodiments, the histone deacetylase inhibitor is administered prior to the administration of the compound which is both an androgen receptor antagonist and a 17-alpha-hydroxylase-C17,20-lyase inhibitor. In even further other certain embodiments, the histone deacetylase inhibitor is administered subsequent to the administration of the compound which is both an androgen receptor antagonist and a 17-alpha-hydroxylase-C17,20-lyase inhibitor.

[0024] In certain embodiments, the histone deacetylase inhibitor and the compound which is both an androgen receptor antagonist and a 17-alpha-hydroxylase-C17,20-lyase inhibitor act synergistically.

[0025] In other certain embodiments, the invention is drawn to a method of treating a refractory prostate cancer comprising the step of administering to a subject having or suspected of having the cancer, SAHA or romidepsin and 3beta-hydroxy-17-(1H-benzimidazole-1-yl)androsta-5,16-diene, in a combined effective amount to treat the cancer.

[0026] In certain embodiments, the invention is drawn to a method of diagnosing prostate cancer or benign prostatic hyperplasia comprising administering to a patient to be diagnosed, a histone deacetylase inhibitor and a compound which is both an androgen receptor antagonist and a 17-alpha-hydroxylase-C17,20-lyase inhibitor, in a combined amount effective to induce sloughing of prostate cells into the urine or seminal fluid, obtaining a sample of said prostate cells in the urine or seminal fluid, and analyzing the sample for the presence of cancer cells. In specific embodiments, the histone deacetylase inhibitor is SAHA or romidepsin. In other specific embodiments, the compound which is both an androgen receptor antagonist and a 17-alpha-hydroxylase-C17,20-lyase inhibitor is 3beta-hydroxy-17-(1H-benzimidazole-1-yl)androsta-5,16-diene.

[0027] The foregoing has outlined rather broadly the features and technical advantages of the present invention in order that the detailed description of the invention that follows may be better understood. Additional features and advantages of the invention will be

described hereinafter which form the subject of the claims of the invention. It should be appreciated by those skilled in the art that the conception and specific embodiment disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present invention. It should also be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope of the invention as set forth in the appended claims. The novel features which are believed to be characteristic of the invention, both as to its organization and method of operation, together with further objects and advantages will be better understood from the following description when considered in connection with the accompanying figures. It is to be expressly understood, however, that each of the figures is provided for the purpose of illustration and description only and is not intended as a definition of the limits of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] For a more complete understanding of the present invention, reference is now made to the following descriptions taken in conjunction with the accompanying drawings.

[0029] FIG. 1A-FIG. 1D show that ER-breast cancer cells lines incubated with HDACi (SAHA or MS-275) reduced cell viability.

[0030] FIG. 2 shows that in the letrozole resistant cells HDACi upregulates ERα.

[0031] FIG. 3 demonstrates that aromatase activity in MDA-MB-231 cells.

[0032] FIG. 4 shows that HDACis stimulate aromatase activity.

[0033] FIG. 5 demonstrates that HDACis upregulate ER and aromatase protein expression.

[0034] FIG. 6 shows cell viability following delivery of a combination of letrozole with HDACi.

[0035] FIG. 7 shows the structure for 3beta-hydroxy-17-(1H-benzimidazole-1-yl)androsta-5,16-diene (VN/124-1)

[0036] FIG. 8A-FIG. 8B shows cell viability following delivery of a combination of SAHA and 3beta-hydroxy-17-(1H-benzimidazole-1-yl)androsta-5,16-diene (VN/124-1).

[0037] FIG. 9A-FIG. 9B shows cell viability following delivery of a combination of SAHA and bicalutamide.

[0038] FIG. 10 shows cell viability following delivery of a combination of SAHA in the presence and absence of androgen.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

[0039] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. For purposes of the present invention, the following terms are defined below.

[0040] As used herein, the use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one." Some embodiments of the invention may consist of or consist essentially of one or more elements, method steps, and/or methods of the invention. It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein.

[0041] As used herein, "about" refers to a numeric value, including, for example, whole numbers, fractions, and percentages, whether or not explicitly indicated. The term "about" generally refers to a range of numerical values (e.g., +/- 5-10% of the recited value) that one of ordinary skill in the art would consider equivalent to the recited value (e.g., having the same function or result). In some instances, the term "about" may include numerical values that are rounded to the nearest significant figure.

[0042] The term "acquired resistance" as used herein refers to resistance that is acquired after at least one treatment with a given agent. Prior to the at least one treatment, the disorder does not possess a resistance to the agent (and, as such, the disorder responds to the first treatment as would a non-resistant disorder). For example, a hormone-resistant cancer is one that initially responds to at least one treatment of a hormone or endrocrine therapy and thereafter develops a resistance to subsequent treatments of the hormone or endrocrine therapy.

[0043] The term "androgen receptor antagonist" as used herein refers to inhibition (partial or complete) of binding between an adrogen and an androgen receptor. An adrogen receptor antagonist includes, for example, a small molecule, or other type of drug or drug-like compound or agent. Examples of an androgen receptor antagonist include, for example, bicalutamide, flutamide, and cyproterone acetate.

[0044] The term "de novo resistance" as used herein refers to resistance that exists prior to treatment with a given agent. Therefore, *de novo* hormone-resistant cancers are resistant to hormone or endocrine therapy prior to the administration of at least one treatment of a hormone or endrocine therapy. In some embodiments, a cancer that is *de novo* resistant to hormone or endocrine therapy is a cancer that is hormone receptor negative (e.g., estrogen receptor negative, progesterone receptor negative or androgen receptor negative).

[0045] The term "effective amount" or "therapeutically effective amount" as used herein is defined as an amount of the agent that will decrease, reduce, inhibit or otherwise abrogate the growth of a neoplasm, induce apoptosis, inhibit angiogenesis of a neoplasm, inhibit metastasis, or induce cytotoxicity in a neoplasm. Thus, an effective amount is an amount sufficient to detectably and repeatedly ameliorate, reduce, minimize or limit the extent of the disease or its symptoms.

[0046] The term "disease-free survival" as used herein is defined as a time between the first diagnosis and/or first surgery to treat a cancer patient and a first reoccurrence. For example, a disease-free survival is "low" if the cancer patient has a first reoccurrence within five years after tumor resection, and more specifically, if the cancer patient has less than about 55 % disease-free survival over 5 years. For example, a high disease-free survival refers to at least about 55% disease-free survival over 5 years.

[0047] The term "endocrine-regulated cancer" as used herein refers to cancers that progress, at least at some stage of their progression, in a manner dependent on the expression of a hormone or a hormone receptor, including, by way of non-limiting example, estrogen, progesterone, androgen and/or the receptors thereof.

[0048] The term "hormone-resistant cancer" as used herein refers to a cancer that has a decreased or eliminated response to a hormone therapy or endocrine therapy when compared to a non-hormone-resistant cancer. From a biological and clinical standpoint, several

patterns of resistance can be distinguished: A) tumors that are inherently insensitive to endocrine receptor (e.g., estrogen receptor or androgen receptor) targeting despite endocrine receptor expression (pan-endocrine therapy resistance or de novo resistance); B) tumors that are hormone dependent but resistant to one or more specific endocrine therapies (agent-selective resistance; for example responded to tamoxifen but not aromatase inhibitor); and C) tumors that initially respond to endocrine therapy but subsequently progress (acquired resistance). All types of resistance are included herein. In some embodiments, the hormone-resistant cancer is a cancer that is hormone-resistant prior to the administration of a hormone or endrocine therapy (i.e., it is *de novo* hormone-resistant). In other embodiments, the hormone-resistant cancer is a cancer that is initially not hormone-resistant, but becomes hormone-resistant after at least one treatment of a hormone or endocrine therapy.

[0049] The term "hormone therapy" or "endocrine therapy" as used herein is defined as a treatment pertaining to blocking or removing hormones. The treatment may remove the gland that synthesizes the hormone or the prohormone, block or inhibit hormone synthesis, or prevent or inhibit the hormone from binding to its receptor, or down-regulate or degrade the hormone receptor. A hormone therapy of the present invention comprises administration of a compound that is capable of preventing or inhibiting the biologic effects of androgens, including, for example, spironolactone (Aldactone, Spiritone), cyproterone acetate (Androcur, Climen, Diane 35, Ginette 35), flutamide (Eulexin), nilutamide (Anandron, Nilandron), bicalutamide (Casodex), ketoconazole (Nizoral), finasteride (Proscar, Propecia), dutasteride (Avodart), and abiraterone.

[0050] The term "hormone agent" or "targeted hormone agent" or "hormone targeted drug" is an agent that blocks or inhibits hormone synthesis, prevents or inhibits the hormone from binding to its receptor, or down-regulates or degrades the hormone receptor. In the present invention, the "hormone agent" or "targeted hormone agent" or "hormone targeted drug" is a compound which is an androgen receptor antagonist and a 17alpha-hydroxylase-C17,20-lyase inhibitor, a 17alpha-hydroxylase-C17,20-lyase inhibitor and a 5-alpha-reductase inhibitor, a 5-alpha-reductase inhibitor, or a combination of the foregoing including, but is not limited to, those agents described in US Pat. No. 5,264,427; US Pat. No. 5,994,334; US Pat. No. 5,994,335; US Pat. No. 6,133,280; US Pat. No. 6,444,683; International Application No. PCT/US2006/07143; International Application No. PCT/US1998/001569; Canadian Pat.

Application No. 2,279,971; Japanese Pat. Application No. 10-533015; and European Pat. Application No. 98 903 769.2, or any combination thereof. In particular embodiments, the "hormone agent" or "targeted hormone agent" or "hormone targeted drug" is a compound which is both an androgen receptor antagonist and a 17alpha-hydroxylase-C17,20-lyase inhibitor. In further particular embodiments, a compound which is both an androgen receptor antagonist and a 17alpha-hydroxylase-C17,20-lyase inhibitor is VN/124-1.

[0051] The term "hyperplasia disease" as used herein is defined as a condition comprising an abnormal increase in the number of cells, including benign hyperplasia, in an organ or tissue. In certain embodiments, the organ or tissue includes a reproductive organ or tissue, including, for example, prostate, testicles, seminal vesicles, breast, uterus, ovaries, and cervix. In particular embodiments, a hyperplasia disease is begnin prostatic hyperplasia. In other embodiments, a hyperplasia disease is a refractory cancer, which may be, for example, a prostate refractory cancer.

[0052] The term "endocrine therapy-resistant" or "hormone resistant" as used herein is defined as a subject receiving an endocrine therapy or hormonal therapy and lacks demonstration of a desired physiological effect, such as a therapeutic benefit, from the administration of the therapy.

[0053] The term "neoplasm" as used herein refers to an abnormal formation of tissue, for example, a tumor. One of skill in the art realizes that a neoplasm encompasses benign tumors and/or malignant tumors. Yet further, as used herein the terms "neoplasm" and "tumor" are interchangeable.

[0054] The term "non-androgen responsive" or "androgen resistant" or "androgen negative" refers to a neoplasm that does not utilize an androgen or a derivative thereof or is not sensitive to an androgen or derivative thereof to develop, proliferative and/or metastasize.

[0055] As used herein the term "refractory" means a cancer that does not respond to treatment. The cancer may be resistant at the beginning of treatment or it may become resistant during treatment.

[0056] The term "subject" as used herein, is taken to mean any mammalian subject to which a composition of the present invention is administered according to the methods

described herein. In a specific embodiment, the methods of the present invention are employed to treat a human subject. Another embodiment includes treating a human subject suffering from a prostate or breast neoplasm.

[0057] The terms "synergistic" or "synergistically" as used herein refer to two or more compounds providing a therapeutic effect that is greater than the sum of the therapeutic effects of the two compounds provided as therapy alone.

[0058] The term "therapeutic benefit" as used herein refers to anything that promotes or enhances the well-being of the subject with respect to the medical treatment of his condition, which includes treatment of pre-cancer, cancer, and hyperproliferative diseases. A list of nonexhaustive examples of this includes extension of the subject's life by any period of time, decrease or delay in the neoplastic development of the disease, decrease in hyperproliferation, reduction in tumor growth, delay of metastases, reduction in cancer cell or tumor cell proliferation rate, and a decrease in pain to the subject that can be attributed to the subject's condition. In a specific embodiment, a therapeutic benefit refers to reversing de novo hormone therapy-resistance or preventing the patient from acquiring an hormone therapy-resistance

II. Methods of Treatment

[0059] In a particular aspect, the present invention provides methods for the treatment of hormone resistant cancers. The present invention relates to a histone deacetylase inhibitor (HDACi) and targeted hormone agents or drugs that shows unexpected, potent synergistic anti-cancer activity. The HDACi increases the sensitivity of the cells to the targeted hormone agent, thus, this combination of HDACi and traditional hormone therapy can be used to treat cancers that are typically not treatable with hormone therapy. Thus, the present invention provides a treatment for hormone resistant cancers in a subject.

[0060] In a further aspect, a targeted hormone agent or drug is a compound which is both an androgen receptor antagonist and a 17alpha-hydroxylase-C17,20-lyase inhibitor. In an even further aspect, the compound which is both an androgen receptor antagonist and a 17alpha-hydroxylase-C17,20-lyase inhibitor is VN/124-1.

A. Cancer Types

[0061] In certain embodiments, the HDACi and a targeted hormone agent or drug are administered to a cell. Cells that are encompassed by the present invention include, but are

not limited to prostate cells or breast cells. More specifically, the breast or prostate cell is a cancer cell, a non-cancerous cell or a benign hyperplastic cell. A prostate or breast cancer cell may include, cells that are drug-resistant, primary cancer cells and/or metastatic cancer cells.

1. Breast Cancer

[0062] Certain embodiments include methods for treating or inhibiting the development of breast cancer in a subject at risk, treating or inhibiting breast cancer metastasis in a subject with primary breast cancer, and/or treating or inhibiting breast cancer progression. Also within the scope of the invention is a method of treating benign breast hyperplasia in a human subject afflicted with benign breast hyperplasia comprising administering a HDACi and a targeted hormone agent thereof to the subject in an amount and duration sufficient to result in cell killing or decreases in cell viability.

2. Prostate Cancer

[0063] In certain aspects, an effective amount of a HDACi and a targeted hormone agent, e.g., a compound which is both an androgen receptor antagonist and a 17alpha-hydroxylase-C17,20-lyase inhibitor, may be administered to a subject suffering from prostate cancer, more specifically, recurrent prostate cancer, more specifically, hormone resistant prostate cancer. The effectiveness of the therapy according to the present invention can be determined in the treatment of prostate cancer by diagnostic methods that are known and used in the art, for example, but not limited to, analysis of prostate specific antigen (PSA), a prostate biopsy, a rectal exam, or analysis of PSA and rectal exam.

[0064] Other embodiments include methods for inhibiting development of prostate cancer in a subject at risk, inhibiting prostate cancer metastasis in a subject with primary prostate cancer, and/or inhibiting prostate cancer progression in subjects.

[0065] Also within the scope of the invention is a method of treating benign prostate hyperplasia in a human subject afflicted with benign prostate hyperplasia comprising administering a HDACi and a targeted hormone agent thereof to the subject in an amount and duration sufficient to result in, for example, cell killing or decreases in cell viability. Benign prostatic hyperplasia or benign prostatic hypertrophy (also referred to as BPH) is a condition characterized by overgrowth of prostate tissue that, for example, pushes against the urethra and/or the bladder thereby blocking the flow of urine. Treating benign prostatic hyperplasia or

benign prostatic hypertrophy encompasses, for example, methods that reduce prostate tissue, reduce the rate of overgrowth of prostate tissue, or facilitate the maintenance of prostate tissue growth (e.g., inhibit further overgrowth). The levels of prostate specific antigen (PSA) produced by the hyperplastic cells could also be stabilized or reduced upon treatment with a HDACi and a targeted hormone agent. In particular embodiments, the targeted hormone agent is a compound which is both an androgen receptor antagonist and a 17alpha-hydroxylase-C17,20-lyase inhibitor. In further embodiments, the compound which is both an androgen receptor antagonist and a 17alpha-hydroxylase-C17,20-lyase inhibitor is VN/124-1.

[0066] Still further, other embodiments can include a method of increasing the sensitivity of androgen negative cancer cells or cancer cells that are resistant to hormone therapy by administering an HDACi. By administering the HDACi to these androgen negative cancer cells or cancer cells that are resistant to hormone therapy, the HDACi, without being bound by theory, increases expression of androgen receptors thereby increasing the sensitivity of these cells to hormone therapy.

[0067] Still other embodiments can include a method of increasing the sensitivity of androgen positive cancer cells by administering an HDACi. By administering the HDACi to these androgen positive cancer cells, the HDACi down regulates expression of the androgen receptors thereby increasing the sensitivity of these cells to hormone therapy. In some embodiments, the cancer cells are androgen positive and the HDACi resensitizes the cells to androgen synthesis inhibitors such as a compound which is both an androgen receptor antagonist and a 17alpha-hydroxylase-C17,20-lyase or a luteinizing hormone-releasing hormone ("LHRH") agonist. Non-limiting examples of LHRH agonists include leuprolide, goserelin, and triptorelin.

B. HDACi

[0068] There are several classes of histone deacetylases (HDACs) including class I HDACs (including, for example, HDAC1, HDAC2, HDAC3, HDAC8, HDAC11), class II HDACs (including, for example, HDAC4, HDAC5, HDAC6, HDAC7, HDAC9, HDAC10), class III HDACs, and class IV HDACs. HDAC inhibitors induce hyperacetylation of histones that modulate chromatin structure and gene expression. These inhibitors also induce growth arrest, cell differentiation, and apoptosis of tumor cells. Recently it was reported that HDAC inhibition restores the expression of functional ERα ER- breast cancer cells (Margueron et al. 2004; Sharma et al. 2006; Yang et al. 2000; Keen et al. 2003). The discovery of recruitment of

HDAC enzymes in cancer has provided a rationale for using inhibition of HDAC activity to release transcriptional repression as viable option toward achieving eventual therapeutic benefit (Vigushin et al. 2002). Histone deacetylase inhibitors (HDACis) block deacetylation function, causing cell cycle arrest, differentiation, and/or apoptosis of many tumors (Vigushin et al. 2002). Silencing of genes that affect growth and differentiation has been shown to occur by aberrant DNA methylation in promoter region and by changes in chromatin structure that involve histone deacetylation. Recent studies have established a link between oncogene-mediated suppression of transcription and recruitment of HDAC into nuclear complex. HDACi such as butyric acid (BA), 4-phenylbutyric acid and trichostatin A reverse this suppression by specific inhibition of HDAC activity, leading to histone hyperacetylation, chromatin relaxation, and enhanced transcription.

[0069] In the present invention, HDAC inhibitors are used to increase the sensitivity or to sensitize hormone resistant cancer cells to hormonal therapy. HDACi include, for example, and can be divided into the following groups: short-chain fatty acid derivatives (e.g., sodium butyrate (Cousens et al., J. Biol. Chem. 254, 1716-1723 (1979)); isovalerate (McBain et al., Biochem. Pharm. 53: 1357-1368 (1997)); valerate (McBain et al., supra); 4-phenylbutyrate (4-PBA) (Lea and Tulsyan, Anticancer Research, 15, 879-873 (1995)); phenylbutyrate (PB) (Wang et al., Cancer Research, 59, 2766-2799 (1999)); propionate (McBain et al., supra); butyramide (Lea and Tulsyan, supra); isobutyramide (Lea and Tulsyan, supra); phenylacetate (Lea and Tulsyan, supra); 3-bromopropionate (Lea and Tulsyan, supra); tributyrin (Guan et al., Cancer Research, 60, 749-755 (2000)); valproic acid, valproate and Pivanex.TM.); hydroxamic acid derivatives (e.g., suberoylanilide hydroxamic acid and derivatives (SAHA) (Richon et al., Proc. Natl. Acad. Sci. USA 95, 3003-3007 (1998)); m-carboxycinnamic acid bishydroxamide (CBHA) (Richon et al., supra); pyroxamide; trichostatin and trichostatin analogues such as trichostatin A (TSA) and trichostatin C (Koghe et al. 1998, Biochem. Pharmacol. 56: 1359-1364); salicylhydroxamic acid (Andrews et al., International J. Parasitology 30, 761-768 (2000)); suberoyl bishydroxamic acid (SBHA) (U.S. Pat. No. 5,608,108); azelaic bishydroxamic acid (ABHA) (Andrews et al., supra); azelaic-1-hydroxamate-9-anilide (AAHA) (Qiu et al., Mol. Biol. Cell 11, 2069-2083 (2000)); 6-(3-chlorophenylureido) carpoic hydroxamic acid (3C1-UCHA); oxamflatin [(2E)-5-[3-[(phenylsufonyl)amino]phenyl]-pent-2-en-4-ynohydroxamic acid] (Kim et al. Oncogene, 18: 2461 2470 (1999)); A-161906, Scriptaid (Su et al. 2000 Cancer Research, 60: 3137-3142); PXD-101 (Prolifix); LAQ-824; CHAP; MW2796 (Andrews et al., supra); MW2996 (Andrews et al., supra); pyroxamide; propenamides; or any of

the hydroxamic acids disclosed in U.S. Pat. Nos. 5,369,108, 5,932,616, 5,700,811, 6,087,367 and 6,511,990); cyclic tetrapeptides and derivatives (e.g., trapoxin and derivatives, including, for example, trapoxin A (TPX)-cyclic tetrapeptide (cyclo-(L-phenylalanyl-L-phenylalanyl-D-pipecolinyl-L-2-amino-8-oxo-9,10-- epoxy decanoyl)) (Kijima et al., J Biol. Chem. 268, 22429-22435 (1993)); FR901228 (FK 228, depsipeptide) (Nakajima et al., Ex. Cell Res. 241, 126-133 (1998)); FR225497 cyclic tetrapeptide (H. Mori et al., PCT Application WO 00/08048 (17 Feb. 2000)); FR901228; apicidin cyclic tetrapeptide [cyclo(N-O-methyl-L-tryptophanyl-L-isoleucinyl-D-pipecolinyl-L-2-amino-8-- oxodecanoyl)] (Darkin-Rattray et al., Proc. Natl. Acad. Sci. USA 93, 1314313147 (1996)); apicidin, including, for example, apicidin Ia, apicidin Ib, apicidin Ic, apicidin IIa, and apicidin IIb (P. Dulski et al., PCT Application WO 97/11366); cyclic-hydroxamic-acid-containing peptides (CHAPs), HC-toxin cyclic tetrapeptide (Bosch et al., Plant Cell 7, 1941-1950 (1995)); WF27082 cyclic tetrapeptide (PCT Application WO 98/48825); WF-3161; Cyl-1 and Cyl-2; diheteropeptin; and chiamydocin (Bosch et al., supra)); benzamides (e.g., MS-275 (MS-27-275) (Saito et al., Proc. Natl. Acad. Sci. USA 96, 4592-4597 (1999)); and 3'-amino derivative of MS-275 (Saito et al., supra); CI-994; MGCD0103; other benzamide analogs); electrophilic ketone derivatives (e.g., trifluoromethyl ketones (Frey et al., Bioorganic & Med. Chem. Lett. (2002), 12, 3443-3447; U.S. Pat. No. 6,511,990) and .alpha.-keto amides such as N-methyl-.alpha.-ketoamides) miscellaneous structures (e.g. psammaplins; depudecin (Kwon et al. 1998. PNAS 95: 3356-3361); organosulfur compounds).

[0070] HDAC inhibitors include those mentioned throughout the specification and those known to one of ordinary skill in the art, and include, but are not limited to, 3-(1-Methyl-4-phenylacetyl-1H-2-pyrrolyl)-N-hydroxy-2-propenamide (APHA Compound 8), Cyclo[(2S)-2-amino-8-oxodecanoyl-1-methoxy-L-tryptophyl-L-isoleucyl-(2R)-2-piperidinexcarbonyl] (Apicidin), Butyric Acid Sodium salt (Sodium Butyrate), 4,5:8,9-Dianhydro-1,2,6,7,11-pentadeoxy-D-threo-D-ido-undeca-1,6-dienitol ((-)-Depudecin), 6-(1,3-Dioxo-1H, 3H-benzo[de]isoquinolin-2-yl)-hexanoic acid hydroxyamide (Scriptaid), 2-[(2-Hydroxynaphthalen-1-ylmethylene)amino]-N-(1-phenethyl)benzamide (Sirtinol), [R-(E,E)]-7-[4-(Dimethylamino)phenyl]-N-hydroxy-4,6-dimethyl-7-oxo-2,4-heptadienamide (Trichostatin A), suberoylanilide hydroxamic acid (SAHA), N-acetyldinaline [4-(acetylamino)-N-(2-amino-phenyl) benzamide] (CI-994), N-(2-Aminophenyl)-4-[N-(pyridine-3-ylmethoxy-carbonyl)aminomethyl]benzamide (MS-275), N-phenyl-N'-(2-Aminophenyl)hexamethylenediamide, romidepsin, belinostat, LAQ-824, LBH-589, MGCD-

0103, KD-5170, depsipeptide, FK228, pivaloyloxymethyl butyrate (Pivanex), HDACi described in US Pat. Application Publication Nos. 20080119424, 20080085902, 20070060614 and US Patent Nos. 6,673,587, 6,706,686, and those identified by methods for identifying HDACi, including, for example, methods described in US Pat. Application Publication No. 20080057529; and any combination thereof. In specific embodiments the HDACi, which are commerically available from Sigma-Aldrich Co. (St. Louis, MO) can include, SAHA, CI-994, MS-275, 3-(1-Methyl-4-phenylacetyl-1H-2-pyrrolyl)-N-hydroxy-2-propenamide (APHA), apicidin, sodium butyrate, (-)-depudecin, scriptaid, sirtinol, and trichostatin A.

C. Treatment Regimen

[0071] Treatment methods will involve treating an individual with an effective amount of a HDACi and a targeted hormone agent or drug. An effective amount is described, generally, as that amount sufficient to detectably and repeatedly to ameliorate, reduce, minimize or limit the extent of a disease or its symptoms. More specifically, it is envisioned that the treatment with the HDACi and targeted hormone agent will kill cells, inhibit cell growth, inhibit metastasis, decrease tumor size and otherwise reverse or reduce the malignant phenotype of tumor cells.

[0072] An effective amount of an HDACi and a targeted hormone agent or drug that may be administered to a cell includes a dose of about 0.001μM to about 1000 μM; about 0.01μM to about 1000 μM; about 0.1μM to about 1000 μM; about 0.001μM to about 100 μM; about 0.01μM to about 100 μM; about 0.1μM to about 100 μM; about 0.001μM to about 10 μM; about 0.01μM to about 10 μM; about 0.1μM to about 10 μM; about 0.001μM to about 1 μM; about 0.01μM to about 1 μM; and about 0.1μM to about 1 μM. Additionally, doses of an HDACi and a target hormone agent or drug to be administered are from about about about 0.01μM to about 0.1 μM; about 0.01μM to about 0. 2 μM; about 0.01μM to about 0.3 μM; about 0.01μM to about 0.4 μM; about 0.01μM to about 0.5 μM; about 0.01μM to about 0.6 μM; about 0.01μM to about 0.7 μM; about 0.01μM to about 0.8 μM; about 0.01μM to about 0.9 μM; 0.001μM to about 0.01 μM; about 0.001μM to about 0.02 μM; about 0.001μM to about 0.03 μM; about 0.001μM to about 0.04 μM; about 0.001μM to about 0.05 μM; about 0.001μM to about 0.06 μM; about 0.001μM to about 0.07 μM; about 0.001μM to about 0.08 μM; about 0.001μM to about 0.09 μM; about 0.001μM to about 0.1 μM; about 0.001μM to about 0.2 μM; about 0.001μM to about 0.3 μM; about 0.001μM to about 0.4 μM; about 0.001μM to about 0.5

μM; about 0.001μM to about 0.6 μM; about 0.001μM to about 0.7 μM; about 0.001μM to about 0.8 μM; about 0.001μM to about 0.9 μM; about 0.001μM to about 1 μM; about 1μM to about 2 μM; about 1μM to about 3 μM; about 1μM to about 4 μM; about 1 μM to about 5 μM; about 1μM to about 6 μM; about 1μM to about 7 μM; about 1μM to about 8 μM; about 1μM to about 9 μM; about 1μM to about 10 μM; about 5 μM to about 10 μM; about 10 μM to about 15 μM; about 15 μM to about 20 μM; about 20 μM to about 30 μM; about 30 μM to about 40 μM; about 40 μM to about 50 μM; about 50 μM to about 60 μM; about 60 μM to about 70 μM; about 70 μM to about 80 μM; about 80 μM to about 90 μM; and about 90 μM to about 100 μM. Of course, all of these amounts are exemplary, and any amount in-between these points is also expected to be of use in the invention.

[0073] The effective amount or "therapeutically effective amounts" of the HDACi and a targeted hormone agent or drug to be used are those amounts effective to produce beneficial results, particularly with respect to cancer treatment, in the recipient animal or patient. Such amounts may be initially determined by reviewing the published literature, by conducting in vitro tests or by conducting metabolic studies in healthy experimental animals. Before use in a clinical setting, it may be beneficial to conduct confirmatory studies in an animal model, preferably a widely accepted animal model of the particular disease to be treated. Preferred animal models for use in certain embodiments are rodent models, which are preferred because they are economical to use and, particularly, because the results gained are widely accepted as predictive of clinical value.

[0074] As is well known in the art, a specific dose level of active compounds such as HDACi and a targeted hormone agent or drug any particular patient depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination, and the severity of the particular disease undergoing therapy. The person responsible for administration will determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

[0075] A therapeutically effective amount of HDACi and a targeted hormone agent or drug as a treatment varies depending upon the host treated and the particular mode of

administration. In one embodiment of the invention the dose range of the HDACi and a targeted hormone agent or drug will be about 0.5 mg/kg body weight to about 500 mg/kg body weight. The term "body weight" is applicable when an animal is being treated. When isolated cells are being treated, "body weight" as used herein should read to mean "total cell weight". The term "total weight" may be used to apply to both isolated cell and animal treatment. All concentrations and treatment levels are expressed as "body weight" or simply "kg" in this application are also considered to cover the analogous "total cell weight" and "total weight" concentrations. However, those of skill will recognize the utility of a variety of dosage range, for example, about 1 mg/kg body weight to 450 mg/kg body weight, 2 mg/kg body weight to 400 mg/kg body weight, 3 mg/kg body weight to 350 mg/kg body weight, 4 mg/kg body weight to 300 mg/kg body weight, 5 mg/kg body weight to 250 mg/kg body weight, 6 mg/kg body weight to 200 mg/kg body weight, 7 mg/kg body weight to 150 mg/kg body weight, 8 mg/kg body weight to 100 mg/kg body weight, 9 mg/kg body weight to 50 mg/kg body weight, or 10 mg/kg body weight to 25 mg/kg body weight. Further, those of skill will recognize that a variety of different dosage levels will be of use, for example, about 0.001 mg/kg, 0.01 mg/kg, 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 7.5 mg/kg, 10 mg/kg, 12.5 mg/kg, 15 mg/kg, 17.5 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, 60 mg/kg, 70 mg/kg, 80 mg/kg, 90 mg/kg, 100 mg/kg, 120 mg/kg, 140 mg/kg, 150 mg/kg, 160 mg/kg, 180 mg/kg, 200 mg/kg, 225 mg/kg, 250mg/kg, 275mg/kg, 300 mg/kg, 325 mg/kg, 350 mg/kg, 375 mg/kg, 400 mg/kg, 450 mg/kg, 500 mg/kg, 550 mg/kg, 600 mg/kg, 700 mg/kg, 750 mg/kg, 800 mg/kg, 900 mg/kg, 1000 mg/kg, 1250 mg/kg, 1500 mg/kg, 1750 mg/kg, 2000 mg/kg, 2500 mg/kg, or 3000 mg/kg. Of course, all of these dosages are exemplary, and any dosage in-between these points is also expected to be of use in the invention. Any of the above dosage ranges or dosage levels may be employed for an HDACi and a targeted hormone agent or drug.

[0076] In certain embodiments, administration of the HDACi SAHA, can be, for example, 400 mg or about 400 mg given orally once daily with food (SAHA can also be given without food). If the subject exhibits dose-limiting toxicities to this dose, the dose may be reduced to 300 mg or about 300 mg orally once daily with food. If necessary, the dose may be further reduced to 300 mg or about 300 mg once daily with food for 5 consecutive days each week, or every other day each week. In the present invention, it is comptemplated that the dose of SAHA can be signigicantly reduced (e.g., 300 mg or about 300 mg given orally once daily

with food, 250 mg or about 250 mg given orally once daily with food, 200 mg or about 200 mg given orally once daily with food, 150 mg or about 150 mg given orally once daily with food, 100 mg or about 100 mg given orally once daily with food, 50 mg or about 50 mg given orally once daily with food) while still providing therapeutic benefit when administered in combination with a compound which is both an androgen receptor antagonist and a 17-alpha-hydroxylase-C17,20-lyase inhibitor (e.g., VN/124-1).

[0077] Administration of a HDACi and a targeted hormone agent to a patient or subject will follow general protocols for the administration of chemotherapeutics, taking into account the toxicity, if any. It is expected that the treatment cycles would be repeated as necessary. It also is contemplated that various standard therapies, as well as surgical intervention, may be applied in combination with the described therapy.

[0078] The treatments may include various "unit doses." Unit dose is defined as containing a predetermined quantity of the HDACi and targeted hormone agent calculated to produce the desired responses in association with its administration, i.e., the appropriate route and treatment regimen. The quantity to be administered, and the particular route and formulation, are within the skill of those in the clinical arts. Also of import is the subject to be treated, in particular, the state of the subject and the protection desired. A unit dose need not be administered as a single injection but may comprise continuous infusion over a set period of time.

[0079] According to the present invention, one may treat the cancer by directly injection a tumor with the HDACi and targeted hormone agent. Alternatively, the tumor may be infused or perfused with the composition using any suitable delivery vehicle. Local or regional administration, with respect to the tumor, also is contemplated. More preferably, systemic administration or oral administration may be performed. Continuous administration also may be applied where appropriate, for example, where a tumor is excised and the tumor bed is treated to eliminate residual, microscopic disease. Delivery via syringe or catherization is preferred. Such continuous perfusion may take place for a period from about 1-2 hours, to about 2-6 hours, to about 6-12 hours, to about 12-24 hours, to about 1-2 days, to about 1-2 wk or longer following the initiation of treatment. Generally, the dose of the therapeutic composition via continuous perfusion will be equivalent to that given by a single or multiple injections, adjusted over a period of time during which the perfusion occurs. For tumors of > 4 cm, the volume to be

administered will be about 4-10 ml (preferably 10 ml), while for tumors of < 4 cm, a volume of about 1-3 ml will be used (preferably 3 ml). Multiple injections delivered as single dose comprise about 0.1 to about 0.5 ml volumes.

[0080] In certain embodiments, the tumor being treated may not, at least initially, be resectable. Treatments with a HDACi and a targeted hormone agent may increase the resectability of the tumor due to shrinkage at the margins or by elimination of certain particularly invasive portions. Following treatments, resection may be possible. Additional treatments subsequent to resection will serve to eliminate microscopic residual disease at the tumor site.

III. Combined Cancer Therapy with Other Anticancer Agents

[0081] In the context of the present invention, it is contemplated that HDACi and the targeted hormone agent thereof may be used in combination with an additional therapeutic agent to more effectively treat the cancer. Anticancer agents may include but are not limited to, radiotherapy, chemotherapy, gene therapy, or immunotherapy that targets cancer/tumor cells.

[0082] When an additional therapeutic agent is administered, as long as the dose of the additional therapeutic agent does not exceed previously quoted toxicity levels, the effective amounts of the additional therapeutic agent may simply be defined as that amount effective to inhibit and/or reduce the cancer growth when administered to an animal in combination with the HDACi and the hormonal therapy agents. This may be easily determined by monitoring the animal or patient and measuring those physical and biochemical parameters of health and disease that are indicative of the success of a given treatment. Such methods are routine in animal testing and clinical practice.

[0083] To kill cells, induce cell-cycle arrest, inhibit cell growth, inhibit metastasis, inhibit angiogenesis or otherwise reverse or reduce the malignant phenotype of cancer cells, using the methods and compositions of the present invention, one would generally contact a cell with HDACi and the hormonal therapy agent thereof in combination with an additional therapeutic agent. These compositions would be provided in a combined amount effective to inhibit cell growth and/or induce apoptosis in the cell. This process may involve contacting the cells with HDACi and the hormonal therapy agent thereof in combination with an additional therapeutic agent or factor(s) at the same time. This may be achieved by contacting the cell with a single composition or pharmacological formulation that includes both agents, or by contacting

the cell with two distinct compositions or formulations, at the same time, wherein one composition includes the HDACi and the hormonal therapy agent thereof and the other includes the additional agent.

[0084] Alternatively, treatment with HDACi and the hormonal therapy agent may precede or follow the additional agent treatment by intervals ranging from minutes to weeks. In embodiments where the additional agent is applied separately to the cell, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the agent would still be able to exert an advantageously combined effect on the cell. In such instances, it is contemplated that one would contact the cell with both modalities within about 12-24 hr of each other and, more preferably, within about 6-12 hr of each other, with a delay time of only about 12 hr being most preferred. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

[0085] It also is conceivable that more than one administration of either TG HDACi and the hormonal therapy agent in combination with an additional therapeutic agent such as anticancer agent will be desired. Various combinations may be employed, where HDACi and the hormonal therapy agent thereof is "A" and the additional therapeutic agent is "B", as exemplified below:

[0086] A/B/A B/A/B B/B/A A/A/B B/A/A A/B/B B/B/B/A B/B/A/B

[0087] A/A/B/B A/B/A/B A/B/B/A B/B/A/A B/A/B/A B/A/A/B B/B/B/A

[0088] A/A/A/B B/A/A/A A/B/A/A A/A/B/A A/B/B/B B/A/B/B B/B/A/B

A. Chemotherapeutic Agents

[0089] In some embodiments of the present invention chemotherapy may be administered, as is typical, in regular cycles. A cycle may involve one dose, after which several days or weeks without treatment ensues for normal tissues to recover from the drug's side effects. Doses may be given several days in a row, or every other day for several days, followed by a period of rest. If more than one drug is used, the treatment plan will specify how often and exactly when each drug should be given. The number of cycles a person receives may be determined before treatment starts (based on the type and stage of cancer) or may be flexible, in

order to take into account how quickly the tumor is shrinking. Certain serious side effects may also require doctors to adjust chemotherapy plans to allow the patient time to recover.

[0090] Chemotherapeutic agents that may be used in combination with the present invention, include, but are not limited to cisplatin (CDDP), carboplatin, procarbazine, mechlorethamine, cyclophosphamide, camptothecin, ifosfamide, melphalan, chlorambucil, busulfan, nitrosurea, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicomycin, mitomycin, etoposide (VP16), tamoxifen, raloxifene, estrogen receptor binding agents, gemcitabien, navelbine, farnesyl-protein tansferase inhibitors, transplatinum, 5-fluorouracil and methotrexate, or any analog or derivative variant of the foregoing.

B. Radiotherapeutic Agents

[0091] Radiotherapeutic agents may also be use in combination with the compounds of the present invention in treating a cancer. Such factors that cause DNA damage and have been used extensively include what are commonly known as γ-rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells. Other forms of DNA damaging factors are also contemplated such as microwaves and UV-irradiation. It is most likely that all of these factors effect a broad range of damage on DNA, on the precursors of DNA, on the replication and repair of DNA, and on the assembly and maintenance of chromosomes. Dosage ranges for X-rays range from daily doses of 50 to 200 roentgens for prolonged periods of time (3 to 4 wk), to single doses of 2000 to 6000 roentgens. Dosage ranges for radioisotopes vary widely, and depend on the half-life of the isotope, the strength and type of radiation emitted, and the uptake by the neoplastic cells.

C. Immunotherapeutic Agents

[0092] Immunotherapeutics may also be employed in the present invention in combination with HDACi and the hormonal therapy agent in treating cancer. Immunotherapeutics, generally, rely on the use of immune effector cells and molecules to target and destroy cancer cells. The immune effector may be, for example, an antibody specific for some marker on the surface of a tumor cell. The antibody alone may serve as an effector of therapy or it may recruit other cells to actually effect cell killing. The antibody also may be conjugated to a drug or toxin (chemotherapeutic, radionuclide, ricin A chain, cholera toxin, pertussis toxin, etc.) and serve merely as a targeting agent. Alternatively, the effector may be a

lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a tumor cell target. Various effector cells include cytotoxic T cells and NK cells.

[0093] Generally, the tumor cell must bear some marker that is amenable to targeting, i.e., is not present on the majority of other cells. Many tumor markers exist and any of these may be suitable for targeting in the context of the present invention. Common tumor markers include carcinoembryonic antigen, prostate specific antigen, urinary tumor associated antigen, fetal antigen, tyrosinase (p97), gp68, TAG-72, HMFG, Sialyl Lewis Antigen, MucA, MucB, PLAP, estrogen receptor, laminin receptor, erb B and p155.

D. Inhibitors of Cellular Proliferation

[0094] The tumor suppressor oncogenes function to inhibit excessive cellular proliferation. The inactivation of these genes destroys their inhibitory activity, resulting in unregulated proliferation. The tumor suppressors p53, p16 and C-CAM are described below.

[0095] High levels of mutant p53 have been found in many cells transformed by chemical carcinogenesis, ultraviolet radiation, and several viruses. The p53 gene is a frequent target of mutational inactivation in a wide variety of human tumors and is already documented to be the most frequently mutated gene in common human cancers. It is mutated in over 50% of human NSCLC (Hollstein et al., 1991) and in a wide spectrum of other tumors.

[0096] The p53 gene encodes a 393-amino acid phosphoprotein that can form complexes with host proteins such as large-T antigen and E1B. The protein is found in normal tissues and cells, but at concentrations which are minute by comparison with transformed cells or tumor tissue.

[0097] Wild-type p53 is recognized as an important growth regulator in many cell types. Missense mutations are common for the p53 gene and are essential for the transforming ability of the oncogene. A single genetic change prompted by point mutations can create carcinogenic p53. Unlike other oncogenes, however, p53 point mutations are known to occur in at least 30 distinct codons, often creating dominant alleles that produce shifts in cell phenotype without a reduction to homozygosity. Additionally, many of these dominant negative alleles appear to be tolerated in the organism and passed on in the germ line. Various mutant alleles appear to range from minimally dysfunctional to strongly penetrant, dominant negative alleles (Weinberg, 1991).

[0098] Another inhibitor of cellular proliferation is p16. The major transitions of the eukaryotic cell cycle are triggered by cyclin-dependent kinases, or CDK's. One CDK, cyclin-dependent kinase 4 (CDK4), regulates progression through the G1. The activity of this enzyme may be to phosphorylate Rb at late G1. The activity of CDK4 is controlled by an activating subunit, D-type cyclin, and by an inhibitory subunit, the p16INK4 has been biochemically characterized as a protein that specifically binds to and inhibits CDK4, and thus may regulate Rb phosphorylation (Serrano et al., 1993; Serrano et al., 1995). Since the p16INK4 protein is a CDK4 inhibitor (Serrano, 1993), deletion of this gene may increase the activity of CDK4, resulting in hyperphosphorylation of the Rb protein. p16 also is known to regulate the function of CDK6.

[0099] p16INK4 belongs to a newly described class of CDK-inhibitory proteins that also includes p16B, p19, p21WAF1, and p27KIP1. The p16INK4 gene maps to 9p21, a chromosome region frequently deleted in many tumor types. Homozygous deletions and mutations of the p16INK4 gene are frequent in human tumor cell lines. This evidence suggests that the p16INK4 gene is a tumor suppressor gene. This interpretation has been challenged, however, by the observation that the frequency of the p16INK4 gene alterations is much lower in primary uncultured tumors than in cultured cell lines (Caldas et al., 1994; Cheng et al., 1994; Hussussian et al., 1994; Kamb et al., 1994; Kamb et al., 1994; Mori et al., 1994; Okamoto et al., 1994; Nobori et al., 1995; Orlow et al., 1994; Arap et al., 1995). Restoration of wild-type p16INK4 function by transfection with a plasmid expression vector reduced colony formation by some human cancer cell lines (Okamoto, 1994; Arap, 1995).

[0100] Other genes that may be employed according to the present invention include Rb, mda-7, APC, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, zac1, p73, VHL, MMAC1/PTEN, DBCCR-1, FCC, rsk-3, p27, p27/p16 fusions, p21/p27 fusions, anti-thrombotic genes (e.g., COX-1, TFPI), PGS, Dp, E2F, ras, myc, neu, raf, erb, fms, trk, ret, gsp, hst, abl, E1A, p300, genes involved in angiogenesis (e.g., VEGF, FGF, thrombospondin, BAI-1, GDAIF, or their receptors) and MCC.

E. Regulators of Programmed Cell Death

[0101] Apoptosis, or programmed cell death, is an essential process in cancer therapy (Kerr et al., 1972). The Bcl-2 family of proteins and ICE-like proteases have been demonstrated to be important regulators and effectors of apoptosis in other systems. The Bcl-2

protein, discovered in association with follicular lymphoma, plays a prominent role in controlling apoptosis and enhancing cell survival in response to diverse apoptotic stimuli (Bakhshi et al., 1985; Cleary and Sklar, 1985; Cleary et al., 1986; Tsujimoto et al., 1985; Tsujimoto and Croce, 1986). The evolutionarily conserved Bcl-2 protein now is recognized to be a member of a family of related proteins, which can be categorized as death agonists or death antagonists.

[0102] Members of the Bcl-2 that function to promote cell death such as, Bax, Bak, Bik, Bim, Bid, Bad and Harakiri, are contemplated for use in combination with HDACi and a homonal therapy agent thereof in treating cancer.

F. Surgery

[0103] It is further contemplated that a surgical procedure may be employed in the present invention. Approximately 60% of persons with cancer will undergo surgery of some type, which includes preventative, diagnostic or staging, curative and palliative surgery. Curative surgery includes resection in which all or part of cancerous tissue is physically removed, excised, and/or destroyed. Tumor resection refers to physical removal of at least part of a tumor. In addition to tumor resection, treatment by surgery includes laser surgery, cryosurgery, electrosurgery, and miscopically controlled surgery (Mohs' surgery). It is further contemplated that the present invention may be used in conjunction with removal of superficial cancers, precancers, or incidental amounts of normal tissue.

[0104] Upon excision of part of all of cancerous cells, tissue, or tumor, a cavity may be formed in the body. Treatment may be accomplished by perfusion, direct injection or local application of the area with an additional anti-cancer therapy. Such treatment may be repeated, for example, every 1, 2, 3, 4, 5, 6, or 7 days, or every 1, 2, 3, 4, and 5 weeks or every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. These treatments may be of varying dosages as well.

G. Other agents

[0105] It is contemplated that other agents may be used in combination with the present invention to improve the therapeutic efficacy of treatment. These additional agents include immunomodulatory agents, agents that affect the upregulation of cell surface receptors and GAP junctions, cytostatic and differentiation agents, inhibitors of cell adhesion, or agents that increase the sensitivity of the hyperproliferative cells to apoptotic inducers. Immunomodulatory agents include tumor necrosis factor; interferon alpha, beta, and gamma; IL-

2 and other cytokines; F42K and other cytokine analogs; or MIP-1, MIP-1beta, MCP-1, RANTES, and other chemokines. It is further contemplated that the upregulation of cell surface receptors or their ligands such as Fas / Fas ligand, DR4 or DR5 / TRAIL would potentiate the apoptotic inducing abilities of the present invention by establishment of an autocrine or paracrine effect on hyperproliferative cells. Increased intercellular signaling by elevating the number of GAP junctions would increase the anti-hyperproliferative effects on the neighboring hyperproliferative cell population. In other embodiments, cytostatic or differentiation agents can be used in combination with the present invention to improve the anti-hyperproliferative efficacy of the treatments. Inhibitors of cell adhesion are contemplated to improve the efficacy of the present invention. Examples of cell adhesion inhibitors are focal adhesion kinase (FAKs) inhibitors and Lovastatin. It is further contemplated that other agents that increase the sensitivity of a hyperproliferative cell to apoptosis, such as the antibody c225, could be used in combination with the present invention to improve the treatment efficacy.

IV. Formulations and Routes for Administration

[0106] Where clinical applications are contemplated, it will be necessary to prepare pharmaceutical compositions of HADCi and/or hormone therapy agents, or any additional therapeutic agent disclosed herein in a form appropriate for the intended application. Generally, this will entail preparing compositions that are essentially free of pyrogens, as well as other impurities that could be harmful to humans or animals.

[0107] One will generally desire to employ appropriate salts and buffers to render delivery vectors stable and allow for uptake by target cells. Buffers also will be employed when recombinant cells are introduced into a patient. Aqueous compositions of the present invention in an effective amount may be dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. Such compositions also are referred to as inocula. The phrase "pharmaceutically or pharmacologically acceptable" refers to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the vectors or cells of the present invention, its use in

therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions.

[0108] The composition(s) of the present invention may be delivered orally, nasally, intramuscularly, intraperitoneally, or intratumorally. In some embodiments, local or regional delivery of the composition thereof, alone or in combination with an additional therapeutic agent, to a patient with cancer or pre-cancer conditions will be a very efficient method of delivery to counteract the clinical disease. Similarly, chemo- or radiotherapy may be directed to a particular, affected region of the subject's body. Regional chemotherapy typically involves targeting anticancer agents to the region of the body where the cancer cells or tumor are located. Other examples of delivery of the compounds of the present invention that may be employed include intra-arterial, intracavity, intravesical, intrathecal, intrapleural, and intraperitoneal routes.

[0109] Intra-arterial administration is achieved using a catheter that is inserted into an artery to an organ or to an extremity. Typically, a pump is attached to the catheter. Intracavity administration describes when chemotherapeutic drugs are introduced directly into a body cavity such as intravesical (into the bladder), peritoneal (abdominal) cavity, or pleural (chest) cavity. Agents can be given directly via catheter. Intravesical chemotherapy involves a urinary catheter to provide drugs to the bladder, and is thus useful for the treatment of bladder cancer. Intrapleural administration is accomplished using large and small chest catheters, while a Tenkhoff catheter (a catheter specially designed for removing or adding large amounts of fluid from or into the peritoneum) or a catheter with an implanted port is used for intraperitoneal chemotherapy. Because most drugs do not penetrate the blood/brain barrier, intrathecal chemotherapy is used to reach cancer cells in the central nervous system. To do this, drugs are administered directly into the cerebrospinal fluid. This method is useful to treat leukemia or cancers that have spread to the spinal cord or brain.

[0110] Alternatively, systemic delivery of the chemotherapeutic drugs may be appropriate in certain circumstances, for example, where extensive metastasis has occurred. Intravenous therapy can be implemented in a number of ways, such as by peripheral access or through a vascular access device (VAD). A VAD is a device that includes a catheter, which is placed into a large vein in the arm, chest, or neck. It can be used to administer several drugs simultaneously, for long-term treatment, for continuous infusion, and for drugs that are vesicants,

which may produce serious injury to skin or muscle. Various types of vascular access devices are available.

[0111] The active compositions of the present invention may include classic pharmaceutical preparations. Administration of these compositions according to the present invention will be via any common route so long as the target tissue is available via that route. This includes but is not limited to, oral, nasal, or buccal routes. Alternatively, administration may be by orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection. Such compositions would normally be administered as pharmaceutically acceptable compositions, described supra. The drugs and agents also may be administered parenterally or intraperitoneally. The term "parenteral" is generally used to refer to drugs given intravenously, intramuscularly, or subcutaneously.

[0112] Solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions also can be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[0113] The therapeutic compositions of the present invention may be administered in the form of injectable compositions either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. These preparations also may be emulsified. A typical composition for such purpose comprises a pharmaceutically acceptable carrier. For instance, the composition may contain 10 mg, 25 mg, 50 mg or up to about 100 mg of human serum albumin per milliliter of phosphate buffered saline. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyloleate. Aqueous carriers include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, etc. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial agents, anti-oxidants, chelating agents and inert gases. The pH, exact concentration of the various components, and the pharmaceutical composition are adjusted according to well known parameters. Suitable excipients for formulation include croscarmellose sodium, hydroxypropyl

methylcellulose, iron oxides synthetic), magnesium stearate, microcrystalline cellulose, polyethylene glycol 400, polysorbate 80, povidone, silicon dioxide, titanium dioxide, and water (purified).

[0114] Additional formulations are suitable for oral administration. Oral formulations include such typical excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. The compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders. When the route is topical, the form may be a cream, ointment, salve or spray.

V. Kits of the Invention

[0115] Any of the compositions described herein may be comprised in a kit. In a non-limiting example, an HDAC inhibitor and/or a compound that is both an androgen receptor antagonist and a 17alpha-hydroxylase-C17,20-lyase inhibitor may be comprised in a kit. The kits may comprise a suitably aliquoted HDAC inhibitor and compound that is both an androgen receptor antagonist and a 17alpha-hydroxylase-C17,20-lyase inhibitor, and the components of the kits may be packaged either in aqueous media or in lyophilized form. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. Where there are more than one component in the kit, the kit also will generally contain a second, third or other additional container into which the additional components may be separately placed. However, various combinations of components may be comprised in a vial. The kits of the present invention also will typically include a means for containing the HDAC inhibitor and/or compound that is both an androgen receptor antagonist and a 17alpha-hydroxylase-C17,20-lyase inhibitor and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow molded plastic containers into which the desired vials are retained.

[0116] Irrespective of the number and/or type of containers, the kits of the invention may also comprise, and/or be packaged with, an instrument for assisting with the injection/administration and/or placement of a component of the kit within the body of an animal or a cell therefrom. Such an instrument may be a syringe, pipette, forceps, and/or any such medically approved delivery vehicle, for example.

VI. Examples

[0117] The following examples are included to demonstrate particular embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. In addition, it is noted that the methods described in the examples can be used interchangeable between the examples to support recitations and embodiments provided in the specification and the claims that follow. Those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1
Molecular effects of HDACi on ERα signaling in ER- breast cancer cells

Methods:

Cell Viability Assessment using MTT assay:

[0118] MTT assay was performed to measure viability of breast cancer cells after treatment with various test compounds (Sabnis let al. 2005). IC_{50} and IC_{25} values for inhibitors is calculated from the linear regression line of the plot of percentage inhibition versus log inhibitor concentration. These IC_{50} values is used for combination or sequencing studies. The effect of combination or sequence of treatment is be determined at IC_{25} of each agent.

Western immunoblotting for expression of ERα and downstream targets:

[0119] The protein extracts from breast cancer (MDA-MB-231 and SKBr3) cells were subjected to western immunoblotting (Sabnis et al. 2005). Protein expression of ERα were examined following HDACi treatment. Protein express of other ER inducible genes such as c-Myc and PgR can also be examined.

Binding Studies:

[0120] To confirm affinity of ERα for E_2 and AE tamoxifen, binding studies are performed (Long et al. 2002). The ligand used for binding studies is ^3H-E_2. Receptor saturation curve is plotted before fixing the concentration of ^3H-E_2 to be used for competitive binding study with 4-OHT. The saturation curve is plotted with varying concentration of ^3H-E_2 and this enables the inventors to precisely measure the total number of ERαs present inside each cell. The

number of receptors is corrected for total number of cells, since treatment with HDACi may have an effect on cell number. The binding affinity of PDs is also tested with ERα in wild type MCF-7 cells.

ER activity measurement:

[0121] To measure the transcriptional activity of re-expressed ERα in the ER- cells ELISA based ERE activity assay are used. This assay is performed on nuclear extracts of untreated or HDACi pretreated malignant breast cells preparation of nuclear extracts and ERE activity assay is performed as per manufacture's instructions (Panomics).

Cell proliferation in response to estrogens:

[0122] To examine whether pre-treatment with HDACi, restores mitogenic effects of estrogens, non-malignant and malignant breast cells are pre-treated with HDACi followed by treatment with estradiol (E_2) and the viability of cells measured by MTT assay.

Results:

[0123] In this study the inventors have utilized ER- MDA-MB-231 cells. This cell line exhibits no ERα protein expression by western blotting and are refractory to growth inhibitory effects of AEs such as tamoxifen and fulvestrant or AIs such as letrozole, exemestane and anastrozole. In addition, proliferation of these cells was not affected by E_2. On the other hand, these cells were significantly inhibited by HDACis SAHA and MS-275 as shown in FIG. 1A - FIG. 1B. The IC_{50} values for SAHA was 205.1 nM. The IC_{50} value for MS-275 was 81.72 nM.

[0124] Furthermore, letrozole alone did not inhibit growth of MDA-MB-231 cells, when combined with MS-275 (0.1nM) and letrozole was found to synergistically inhibit cell growth in a dose dependent manner (IC_{50}=15.43 nM), as shown in FIG. 1C. FIG. 1D shows that letrozole alone did not inhibit growth of MDA-MB-231 cells, however, when combined with SAHA, the combination was found to synergistically inhibit cell growth in a dose dependent manner. Similar results were also obtained in cells that have acquired resistance to letrozole (LTLT-Ca).

[0125] In addition, protein expression of ERα was up-regulated 9.9 and 8 fold after treatment with HDACis SAHA, MS-275 (10nM) respectively. Although, HDACi BA was not a potent inhibitor of cell growth (IC_{50} = 20.28 mM), ERα protein expression was up-regulated 15

fold after treatment with 1μM BA for 24 hours. This restoration of ERα was also associated with restoration of response to tamoxifen. The combination of HDACi with tamoxifen was significantly better than single agent alone *($p < 0.01$)*.

Example 2
Effects on Aromatase Activity

[0126] Aromatse activity was measured by Yue et al. (1997). In addition, the expression and activation of aromatase was seen after treatment with HDACi. The basal level of aromatase activity in MDA-MB-231 cells was found to be 3.02 pmoles/μg of protein/hour. When treated with MS-275 (1μM) for 24 hours and then incubated with 1β-3H-Androstenedione for 18 hours, the aromatase activity was found to be 15.193 pmoles/μg of protein/hour. This up-regulation of aromatase activity was dose dependent. A similar increase in aromatase activity was observed after pre-treatment with butyric acid. Also, a 24 hour treatment of MDA-MB-231 cells with MS-275 (10nM), SAHA (10nM) and BA (1μM) up-regulated the expression of aromatase by 2.6, 1.77 and 1.2 fold respectively.

Example 3
In vivo dose response effects of HDACi

[0127] Antitumor efficacies of each HDACi are tested in female mice bearing MDA-MB-231 and SKBr3 tumors using in vivo mouse xenograft model. The cell lines used for this study are estrogen independent ER- cell lines. SKBr3 cells are essentially used for studies with AIs, since these cells have endogenous high levels of aromatase, which is inhibited by AIs. This model simulates advanced and ER-, hormone refractory breast cancer, which is usually associated with mortality of the disease. The xenograft studies are performed as described by Long et al. 2004; Takabatake et al. 2007. Each agent is given at 5 different doses (doses will depend on MED as determined in specific aim 2) *po* and *sc* and effect on the growth of tumors will be examined. During the course of experiment, tumors are measured weekly with calipers and tumor volume calculated using the formula $\mathbf{4/3\pi r1^2 r_2}$. After completion of the treatment the animals are euthanized, tumors and uteri are weighed and collected for further analysis. The weight of the uteri is an important bioassay for estrogenic/antiestrogenic activity of the administered agent, since OVX mice have no significant source of estrogen production. The tumors are examined for expression of signaling proteins in the ER pathway as well as activity of ER and downstream targets. The dose of each agent that causes maximum inhibition of tumor growth are used for combination studies.

Example 4
Mouse xenograft studies combination of HDACi with AEs/AIs

[0128] Experiments are performed to confirm the anti-tumor activity of HDACi and AEs/AIs in mouse xenograft model. The mice receive treatment for a period of at least 8 weeks. The groups of treatment *(n ≥ 10)* used in the experiment include (but may not be limited to) the following: **Groups to be tested: 1.**Vehicle treated control, **2.**AE alone (*sc* 5 times a week), **3.**AI alone (*sc* 5 times a week), **4.**HDACi alone, **5.**HDACi *plus* AE, **6.**HDACi *plus* AI, **8.**HDACi and AE in sequence, **9.**HDACi and AI in sequence. The dose, frequency and route of administration for HDACi are determined from the above experiments. The dose of AEs and AIs are used as determined (Lu et al., 1999) and the drugs will be given *sc*. During the course of experiment, tumor volumes are measured weekly. After approximately 6-8 weeks, the animals are euthanized, tumors and uteri will be weighed collected for further analysis. The tumors are examined for expression of signaling proteins in the ER pathway as well as activity of ER and downstream targets.

Example 5
In vivo metastases mouse model

[0129] To examine the effects of the above-mentioned combinations and sequences on metastatic spread, a metastasis mouse model is used (Fulton et al. 2006; Walser et al. 2006). Here, MDA-MB-231 cells are injected intravenously into OVX nude mice. All the above-mentioned agents are administered and after 4 weeks, mice are sacrificed and formation of pleural metastasis are assessed.

Example 6
Molecular effects of HDACi Androgen negataive prostate cancer cells

Methods:

Cell Viability Assessment using MTT assay:

[0130] MTT assay is performed to measure viability of prostate cancer cells after treatment with various test compounds (Sabnis let al. 2005). IC_{50} and IC_{25} values for inhibitors is calculated from the linear regression line of the plot of percentage inhibition versus log inhibitor concentration. These IC_{50} values is used for combination or sequencing studies. The effect of combination or sequence of treatment is be determined at IC_{25} of each agent.

Western immunoblotting for expression of Androgen receptor and downstream targets:

[0131] The protein extracts from prostate cancer cells are subjected to western immunoblotting (Sabnis et al. 2005). Protein expression of androgen receptor are examined following HDACi treatment.

Androgen activity measurement:

[0132] To measure the transcriptional activity of re-expressed androgen receptor in the androgen receptor negative- cells ELISA based activity assay are used. This assay is performed on nuclear extracts of untreated or HDACi pretreated malignant prostate cells preparation of nuclear extracts and activity assay is performed as per manufacture's instructions (Panomics).

Cell proliferation in response to androgens:

[0133] To examine whether pre-treatment with HDACi, restores mitogenic effects of androgens, non-malignant and malignant prostate cells are pre-treated with HDACi followed by treatment with an androgen and the viability of cells measured by MTT assay.

Example 7
HDAC Inhibitors sensitize ER-negative breast cancer cells to aromatase inhibitors

[0134] The ability of HDACi to sensitive exemplary ER-negative breast cancer cells to aromatase inhibitors was characterized. The following exemplary materials and methods were utilized, although one of skill in the art recognizes that alternative but analogous materials and methods may be employed.

Cell Line and Cell Proliferation Assay

[0135] Exemplary ER negative cell lines (MDA-MB-231) were used. For a cell proliferation assay, 10^4 cells were plated in a 96 well plate and treated with indicated drugs for 6 days. The medium was replaced after 3 days. On day 7, 500μg/ml of MTT solution was added to each well and cells incubated for 3 hours. The tetrazolium dye trapped inside the mitochondria of the cells was dissolved in DMSO and the absorbance was measured at 560nm.

Western Immunoblotting

[0136] Expression of ER and Aromatase proteins was examined by Western blotting. β-actin was used as a loading control.

Radiometric ^3H2O release assay for aromatase activity

[0137] 150,000 cells were plated in IMEM without PR with 5% steroid free serum, 1% penicillin –streptomycin and 750μg/ml G418. Next day the cells were incubated with 0.5μCi

of [1β^{3}H] androstenedione (Specific activity 23.5 Ci/mmole) in 1 ml of media containing 1% charcoal stripped serum for 18 hours. The medium was then collected and treated with TCA (trichloroacetic acid) to precipitate proteins. The residual steroids in the medium were extracted and removed with chloroform and further treated with a 2.5% charcoal suspension. The ^3H_2O in the supernatant was measured using a scintillation counter. In this assay ^3H_2O is released during conversion of [1β^{3}H] Δ4A to estrone, catalyzed by the enzyme aromatase. For pre-treatment studies, cells were plated as described above and then next day pre-treated with indicated agent for 24 hours before incubating with [1β^{3}H] Δ4A for 18 hours. The activity of the enzyme is corrected for protein concentration in the cells plated and treated.

Statistics

[0138] ANOVA was performed for multiple comparisons. All comparisons are two sided and p of less than 0.05 was considered statistically significant.

[0139] The IC_{50} values of HDACis in the exemplary MDA-MB-231 cells is provided in Table 1 below.

[0140] Table 1: IC_{50} values of exemplary HDACis

Compound	IC_{50} Values
SAHA	205.10 nM
MS-275	81.72 nM
BA	2028 mM

[0141] It is demonstrated that the exemplary MDA-MB-231 cells are hormone refractory and ER-negative, but they have detectable levels of basal aromatase activity (FIG. 3). Also, the growth of the MDA-MB-231 cells is inhibited by HDAC inhibitors (SAHA, MS-275 and BA) in a dose dependent manner. Furthermore, HDAC inhibitors up-regulate ER and aromatase protein expression after 24 hour treatment (FIG. 5). The aromatase activity is upregulated by HDAC inhibitors SAHA, MS-275 and BA in a dose dependent manner (FIG. 4). Finally, when combined with HDACi SAHA or MS-275, letrozole inhibits the growth of ER-

negative MDA-MB-231 cells in a dose dependent manner with IC_{50} values of 1.13 μM and 15.43 nM respectively (FIG. 6).

[0142] Therefore, histone deacetylase inhibitors can upregulate ER and aromatase protein expression and upregulate aromatase activity and sensitize ER negative breast cancer cells to endocrine therapy. The combination of AEs or AIs with HDAC inhibitors represents a new strategy for the treatment of cancer, including ER-negative breast cancers that otherwise are treated with chemotherapy only, for example.

Example 8
VN/124-1 in combination with suberoylanilide hydroxamic acid (SAHA)

[0143] The present inventors determine the efficacy of VN/124-1 and SAHA alone and in combination over a vast array of androgen dependent and androgen refractory cell lines. In particular, LNCaP and LNCaP-HP were used. LNCaP cells were isolated from the left supraclavicular lymph node of a 50-year-old Caucasian male with confirmed metastatic prostate cancer. These cells contain a functional AR that has a mutation in its ligand-binding domain. LNCaP-HP cells were derived from the LNCaP parental line in our lab through repeated passaging (>100 passages). These cells exhibit increased AR expression and are resistance to bicalutamide treatment in comparison to the parental cell line. The effects on cell proliferation by VN/124-1 alone and in combination with SAHA can be measured by, for example, the method described above or any other method known by one of ordinary skill in the art. VN/124-1 in combination with SAHA increased cell death when compared to VN/124-1 alone in both LNCaP cells (FIG. 8A) and LNCaP-HP cells (FIG. 8B). Each drug is assessed in the range of, for example, 0.001μM to 100μM to develop a dose response curve. Experiments are carried out 3 times with 6 replicates per dose per experiment (n=18). Results are plotted using the average of each dose over all three experiments and fitted with a best-fit sigmoidal dose response curve. Significant difference between single treatments and combinations for a given dose are calculated using a one-way ANOVA. The isobole method using the equation Ac/Ae+Bc/Be=D is used to determine that the combination is synergistic (Berenbaum, M.C., Adv Cancer Res, 1981. 35: p. 269-335). Ac and Bc represent the concentrations of drugs A and B used in the combination, and Ae and Be represent the concentrations of drugs A and B needed to illicit an equivalent effect when administered alone. If the combination index (D) is less than 1 then the

drugs act synergistically, if it is equal to or greater than 1 then the drugs act in an additive or antagonistic manner, respectively.

Example 9
Bicalutamide in combination with suberoylanilide hydroxamic acid (SAHA)

[0144] The present inventors determine the efficacy of bicalutamide and SAHA alone and in combination over a vast array of androgen dependent and androgen refractory cell lines. In particular, LNCaP and LNCaP-HP were used. LNCaP cells were isolated from the left supraclavicular lymph node of a 50-year-old Caucasian male with confirmed metastatic prostate cancer. These cells contain a functional AR that has a mutation in its ligand-binding domain. LNCaP-HP cells were derived from the LNCaP parental line in our lab through repeated passaging (>100 passages). These cells exhibit increased AR expression and are resistance to bicalutamide treatment in comparison to the parental cell line. The effects on cell proliferation using bicalutamide in combination with SAHA can be measured by, for example, the method described above or any other method known by one of ordinary skill in the art. Bicalutamide in combination with SAHA increased cell death when compared to bicalutamide alone in both LNCaP cells (FIG. 9A) and LNCaP-HP cells (FIG. 9B). Each drug is assessed in the range of, for example, 0.001μM to 100μM to develop a dose response curve. Experiments are carried out 3 times with 6 replicates per dose per experiment (n=18). Results are plotted using the average of each dose over all three experiments and fitted with a best-fit sigmoidal dose response curve. Significant difference between single treatments and combinations for a given dose are calculated using a one-way ANOVA. The isobole method using the equation Ac/Ae+Bc/Be=D is used to determine that the combination is synergistic (Berenbaum, M.C., Adv Cancer Res, 1981. 35: p. 269-335). Ac and Bc represent the concentrations of drugs A and B used in the combination, and Ae and Be represent the concentrations of drugs A and B needed to illicit an equivalent effect when administered alone. If the combination index (D) is less than 1 then the drugs act synergistically, if it is equal to or greater than 1 then the drugs act in an additive or antagonistic manner, respectively.

Example 10

Suberoylanilide hydroxamic acid (SAHA) activity in the presence of androgen

[0145] HP-LNCaP cells were cultured in media containing charcoal stripped FBS with or without the synthetic androgen R1881 (17beta-hydroxy-17alpha-methyl-estra-4,9,11-

trien-3-one) and treated with SAHA (FIG. 10). Viability was measured at 96 hours using an MTT-based assay. In the presence of androgen the IC50 value for SAHA was 417nM whereas in the absence of androgen the IC50 for SAHA was 48nM, demonstrating almost a 9-fold decrease in the IC50 value. These data demonstrate that SAHA exhibits increased sensitivity at lower androgen concentrations. The observation that depriving cells of androgens (which is a mechanism of action of VN/124-1 in vivo) increases the sensitivity of SAHA supports a synergistic combination comprising SAHA and VN/124-1.

Example 11

VN/124-1 in combination with suberoylanilide hydroxamic acid (SAHA) in vivo

[0146] To determine the efficacy of VN/124-1 and SAHA alone and in combination against a vast array of androgen dependent and androgen refractory prostate cells, a nude mouse xenograph model is used as described below. Prostate cancer cells described in the specification and those known to one of ordinary skill in the art (see, for example, Sobel et al. J Urol. 2005 Feb ;173: 342-359; Sobel et al. J Urol. 2005 Feb ;173:360-72) are used in the animal model desribed below. Using the animal model described in this example it is shown that VN/124-1 and SAHA act synergistically in the treatment of prostate cancer in vivo. It is noted that other appropriate and accepted animal models can be used to determine the efficacy of VN/124-1 and SAHA alone and in combination against a vast array of androgen dependent and androgen refractory prostate cells in vivo with the animal model described in this example presented merely for illustrative purposes.

[0147] Briefly, cells are grown in complete medium. When cells reach 70-80% confluence, 3-4 hrs before harvesting, medium is replaced with fresh medium to remove dead and detached cells. Prior to removal of cells, medium is removed and cells are washed with PBS. Cells are most commonly removed from a culture substrate by treatment with trypsin, or trypsin-EDTA, which requires adding a minimum amount of either to the cells in culture so as to minimize damage to cells, followed by dispersing cells and adding complete medium (e.g., 10:1 to 5:1). Following removal of cells from the culture substrate, cells are centrifuged in the cold immediately at or below, for example, 1500 rpm for 2-5 minutes and washed twice with PBS and stored on ice. Cells are counted using, for example, a hemocytometer with dead cells being identified and excluded using, for example, trypan blue stain. Cells are finally suspended in a volume so that 300 mu.L contains the required number of cells per injection (e.g., 3.0 x 10.sup.6

cells per injection). Following the preparation of cells, nude mice, for example, are injected with cells (3.0 x 106) subcutaneously into the lower flank. When tumors reach an appropriate size, for example, a diameter of about 50–60 mm3, the mice are divided into 4 groups: one control group, in which tumors are not treated at all or are injected with phosphate buffered saline (PBS) in equal volumes as treatment groups, a second and a third group, which are administered VN/124-1 and SAHA, respectively, and a fourth group, which is administered a combination of VN/124-1 and SAHA. Animals are monitored over several weeks until control tumors reach, for example, a diameter of about 1 cm, when animals are to be euthanized. Finally, tumor size and constituents are determined.

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[0148] The following references, and those cited in the specficiation, are specifically incorporated herein by reference in their entirety.

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Yue W, and Brodie A J Steroid Biochem Mol Biol, 63:317-328, 1997.

[0149] Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined by the appended claims. Moreover, the scope of the present application is not intended to be limited to the particular embodiments of the process, machine, manufacture, composition of matter, means, methods and steps described in the specification. As one of ordinary skill in the art will readily appreciate from the disclosure of the present invention, processes, machines, manufacture, compositions of matter, means, methods, or steps, presently existing or later to be developed that perform substantially the same function or achieve substantially the same result as the corresponding embodiments described herein may be utilized according to the present invention. Accordingly, the appended claims are intended to include within their scope such processes, machines, manufacture, compositions of matter, means, methods, or steps.

claims

What is claimed is:

1. A method of treating a hyperplasia disease which was originally hormone dependent but has become refractory to hormone therapy, comprising administering to a subject having or suspected of having the hyperplasia disease (i) a histone deacetylase inhibitor and (ii) a compound which is both an androgen receptor antagonist and a 17alpha-hydroxylase-C17,20-lyase inhibitor, in an effective amount to treat the hyperplasia disease.

2. The method of claim 1 wherein the hyperplasia disease is a refractory cancer.

3. The method of claim 1 or 2 wherein the hyperplasia disease is selected from the group consisting of prostate cancer, testicular cancer and cancer of the seminal vesicles.

4. The method of claim 1 or 2 wherein the hyperplasia disease is selected from the group consisting of breast cancer, uterine cancer, ovarian cancer, and cervical cancer.

5. The method of claim 1 wherein the hyperplasia disease is benign prostatic hyperplasia.

6. The method of claim 1, 2, or 3 wherein the hyperplasia disease is prostate cancer.

7. The method of any of the preceding claims wherein the histone deacetylase inhibitor is selected from the group consisting of suberoylanilide hydroxamic acid (SAHA), CI-994, MS-275, 3-(1-Methyl-4-phenylacetyl-1H-2-pyrrolyl)-N-hydroxy-2-propenamide (APHA), apicidin, sodium butyrate, (-)-depudecin, scriptaid, sirtinol, trichostatin A, romidepsin, belinostat, LAQ-824, LBH-589, MGCD-0103, KD-5170, depsipeptide, and any combination thereof.

8. The method of claim 7 wherein the histone deacetylase inhibitor is SAHA.

9. The method of claim 7 wherein the histone deacetylase inhibitor is romidepsin.

10. The method of any of the preceding claims wherein the compound which is both an androgen receptor antagonist and a 17-alpha-hydroxylase-C17,20-lyase inhibitor is 3beta-hydroxy-17-(1H-benzimidazole-1-yl)androsta-5,16-diene.

11. The method of any of the preceding claims further comprising administering an effective amount of an androgen receptor antagonist selected from bicalutamide, flutamide, cyproterone acetate and/or a hormone therapy selected from ketoconazole or abiraterone.

12. The method of any of the preceding claims, wherein the histone deacetylase inhibitor and the compound which is both an androgen receptor antagonist and a 17-alpha-hydroxylase-C17,20-lyase inhibitor are administered simultaneously.

13. The method of any of the preceding claims, wherein the histone deacetylase inhibitor is administered prior to the administration of the compound which is both an androgen receptor antagonist and a 17-alpha-hydroxylase-C17,20-lyase inhibitor.

14. The method of any of the preceding claims, wherein the histone deacetylase inhibitor is administered subsequent to the administration of the compound which is both an androgen receptor antagonist and a 17-alpha-hydroxylase-C17,20-lyase inhibitor.

15. The method of any of the preceding claims, wherein the histone deacetylase inhibitor and the compound which is both an androgen receptor antagonist and a 17-alpha-hydroxylase-C17,20-lyase inhibitor act synergistically.

16. A method of treating a refractory prostate cancer comprising the step of administering to a subject having or suspected of having the cancer (i) SAHA or romidepsin and (ii) 3beta-hydroxy-17-(1H-benzimidazole-1-yl)androsta-5,16-diene, in a combined effective amount to treat the cancer.

17. A method of diagnosing prostate cancer or benign prostatic hyperplasia comprising administering to a patient to be diagnosed a histone deacetylase inhibitor and a compound which is both an androgen receptor antagonist and a 17-alpha-hydroxylase-C17,20-lyase inhibitor, in a combined amount effective to induce sloughing of prostate cells into the urine or seminal fluid, obtaining a sample of said prostate cells in the urine or seminal fluid, and analyzing the sample for the presence of cancer cells.

18. The method of claim 17 wherein the histone deacetylase inhibitor is SAHA or romidepsin.

19. The method of claim 17 or 18 wherein the compound which is both an androgen receptor antagonist and a 17-alpha-hydroxylase-C17,20-lyase inhibitor is 3beta-hydroxy-17-(1H-benzimidazole-1-yl)androsta-5,16-diene.

FIGURES

FIG. 1A

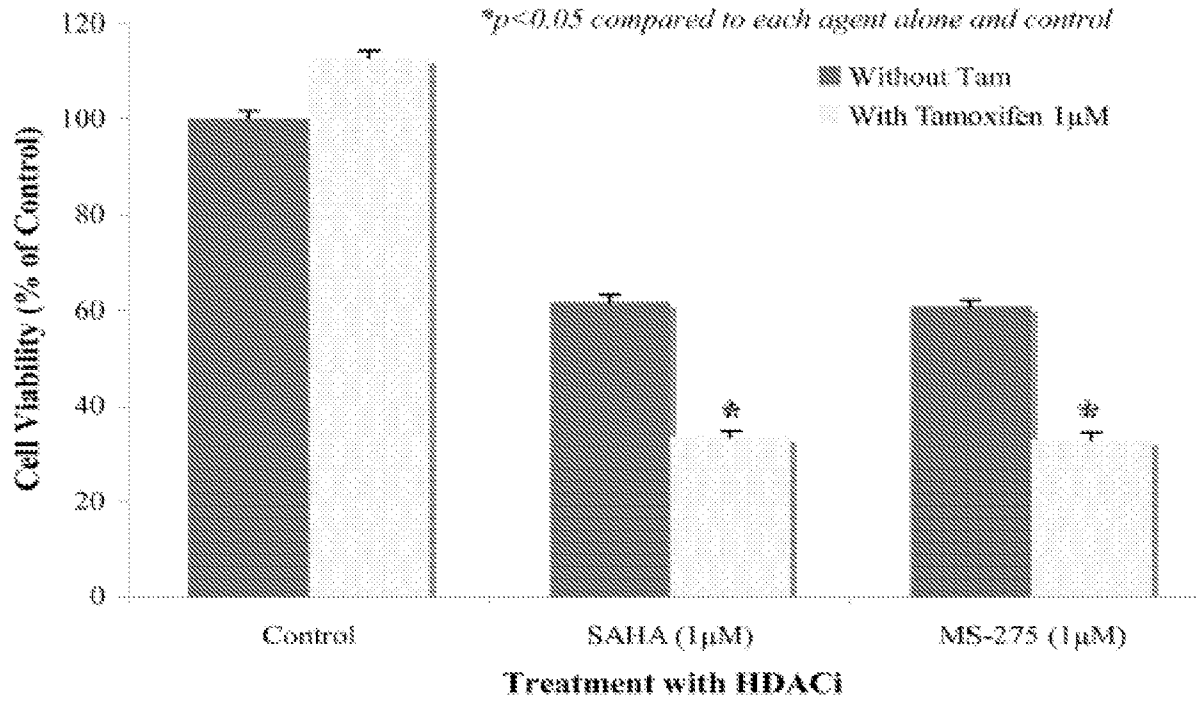


FIG. 1B

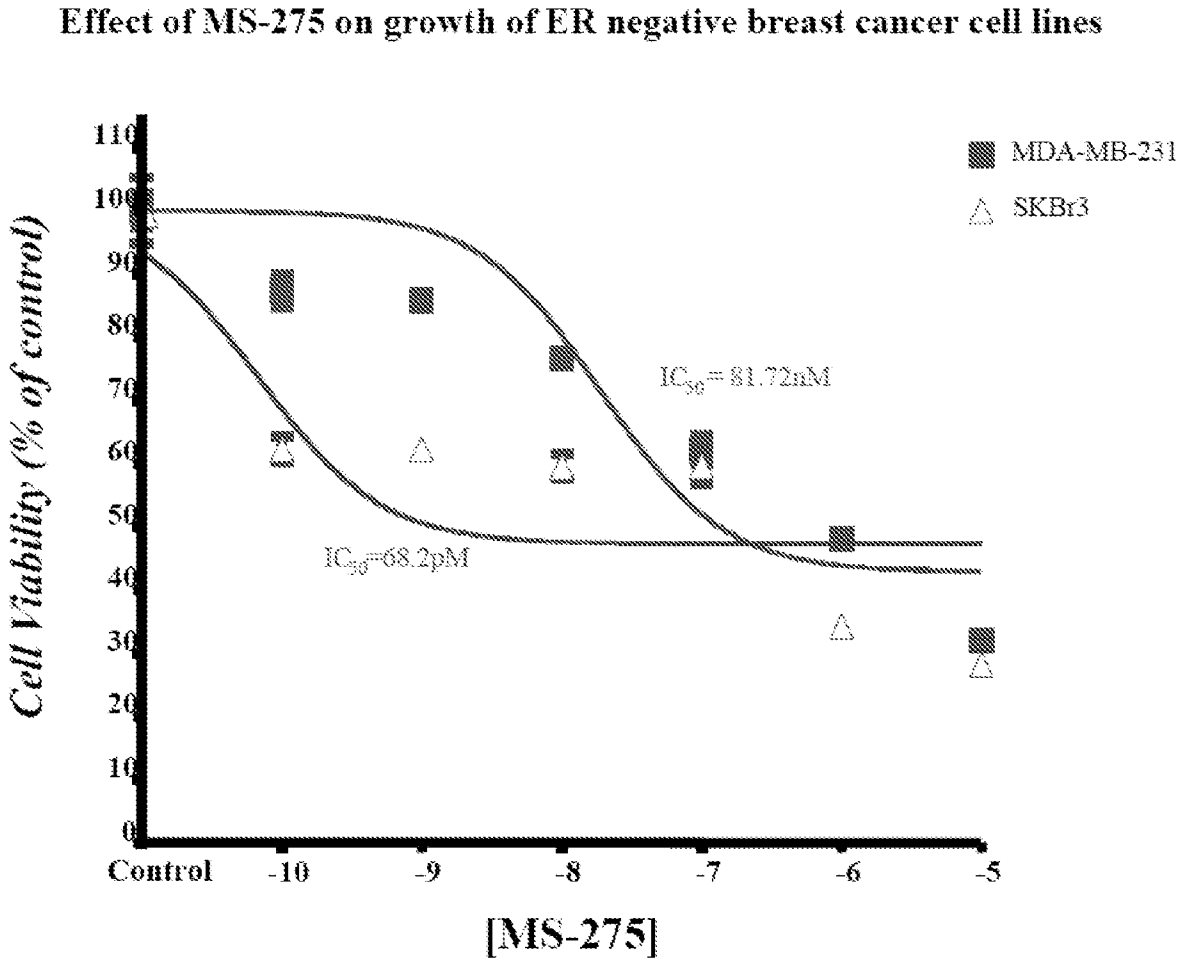

Effect of MS-275 on growth of ER negative breast cancer cell lines
MDA-MB-231
SKBr3
IC50 = 81.72nM
IC50=68.2pM
Cell Viability (% of control)
110
100
90
80
70
60
50
40
30
20
10
0
Control
-10
-9
-8
-7
-6
-5
[MS-275]

FIG. 1C

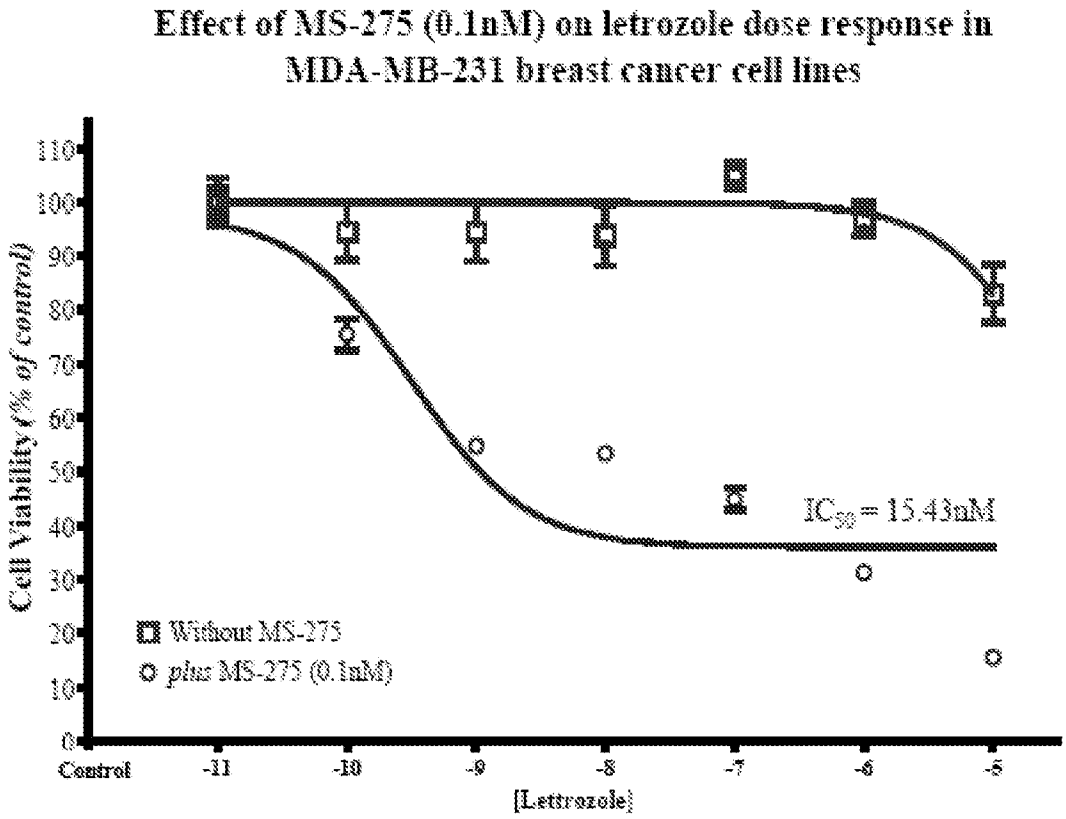

Effect of MS-275 (0.1nM) on letrozole dose response in MDA-MB-231 breast cancer cell lines
Cell Viability (% of control)
110
100
90
80
70
60
50
40
30
20
10
0
IC50 = 15.43nM
Without MS-275
plus MS-275 (0.1nM)
Control
-11
-10
-9
-8
-7
-6
-5
[Letrozole]

FIG. 1D

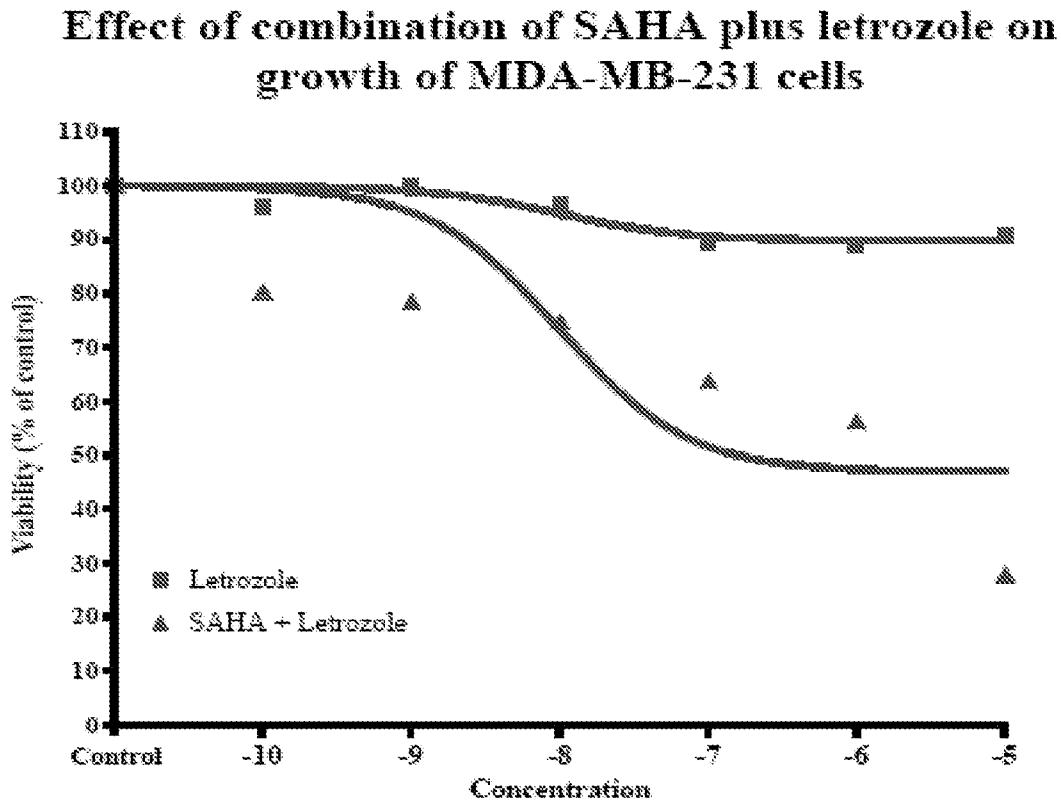

Effect of combination of SAHA plus letrozole on growth of MDA-MB-231 cells
Viability (% of control)
110
100
90
80
70
60
50
40
30
20
10
0
Letrozole
SAHA + Letrozole
Control
-10
-9
-8
-7
-6
-5
Concentration

FIG. 2

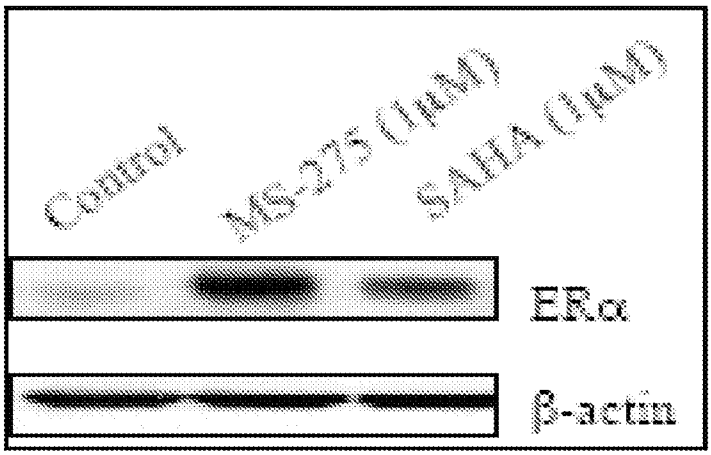


FIG. 3

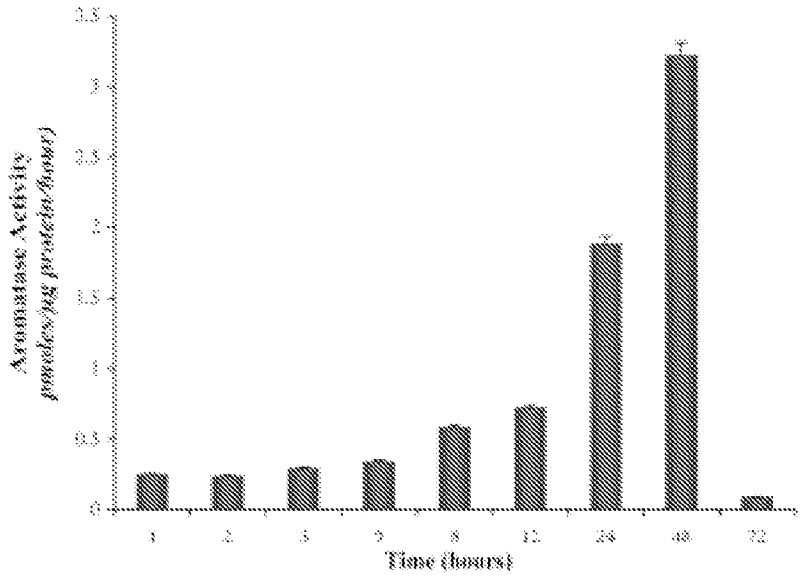


FIG. 4

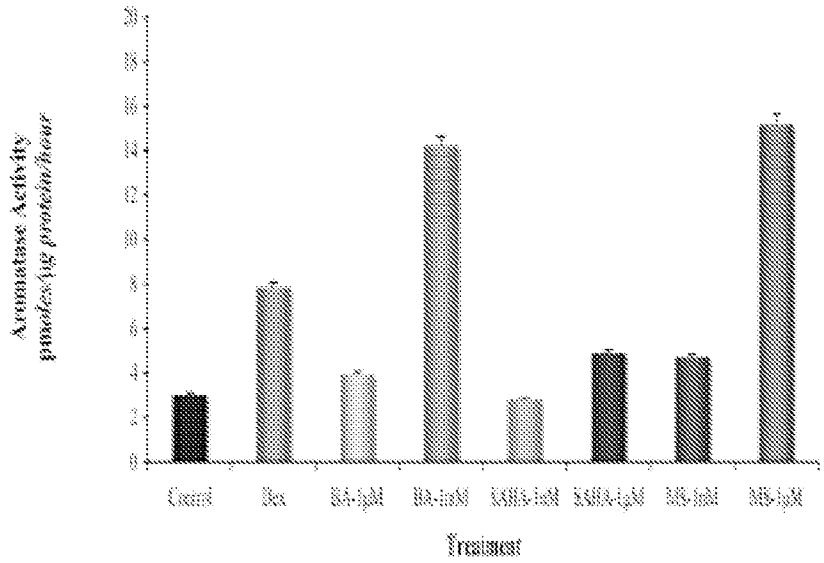

Aromatase Activity
pmoles/µg protein/hour
0
2
4
6
8
10
12
14
16
18
20
Control
Dex
BA-1µM
BA-1mM
SAHA-1mM
SAHA-1µM
MS-1mM
MS-1µM
Treatment

FIG. 5

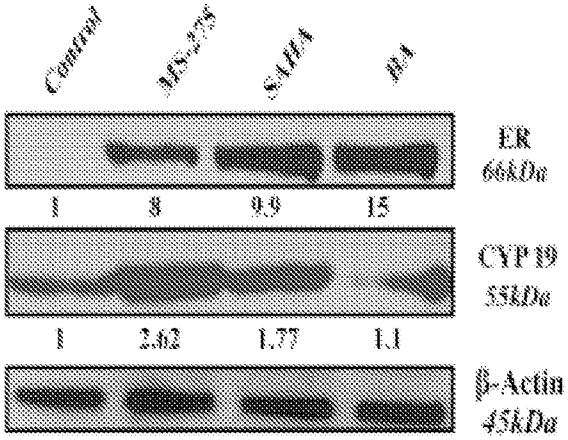

Control
MS-275
SAHA
RA
ER
66kDa
1
8
9.9
15
CYP19
55kDa
1
2.62
1.77
1.1
β-Actin
45kDa

FIG. 6

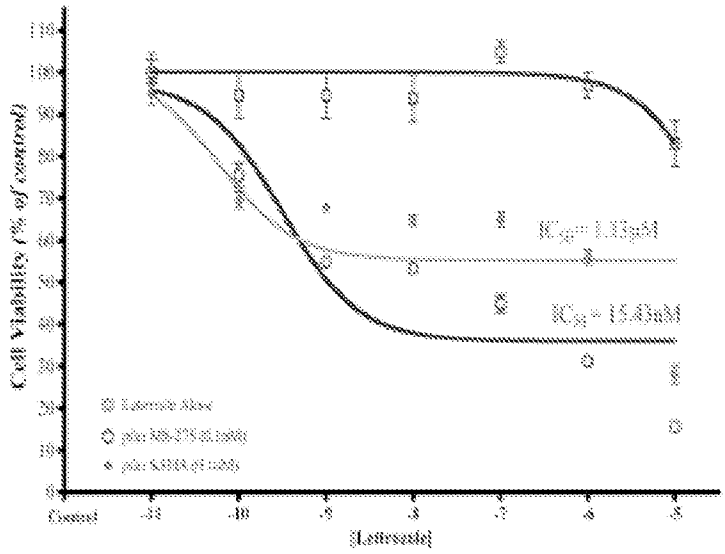
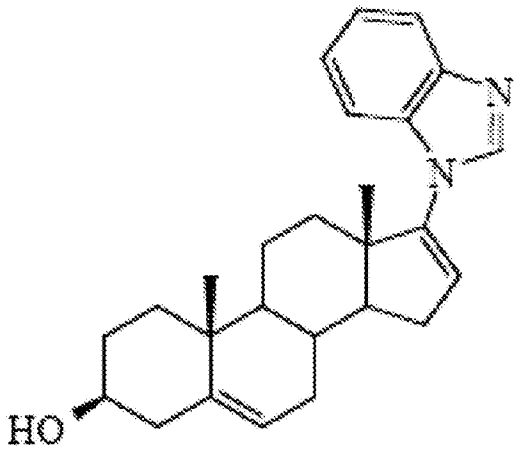

Cell Viability (% of control)
[Letrozole]
Control
Letrozole Alone

FIG. 7



VN/124-1

FIG. 8A

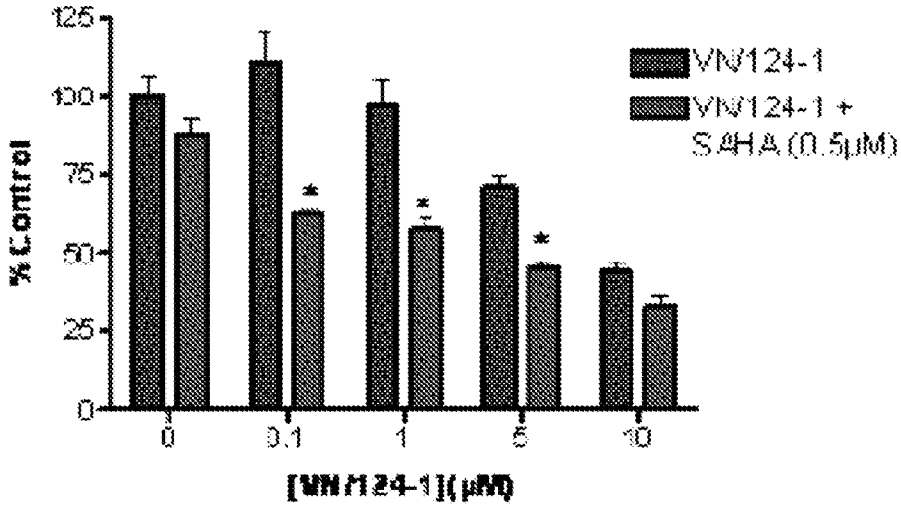

125
100
75
50
25
0
% Control
0
0.1
1
5
10
[VN124-1](μM)
VN124-1
VN124-1 +
SAHA (0.5μM)
*
*
*

FIG. 8B

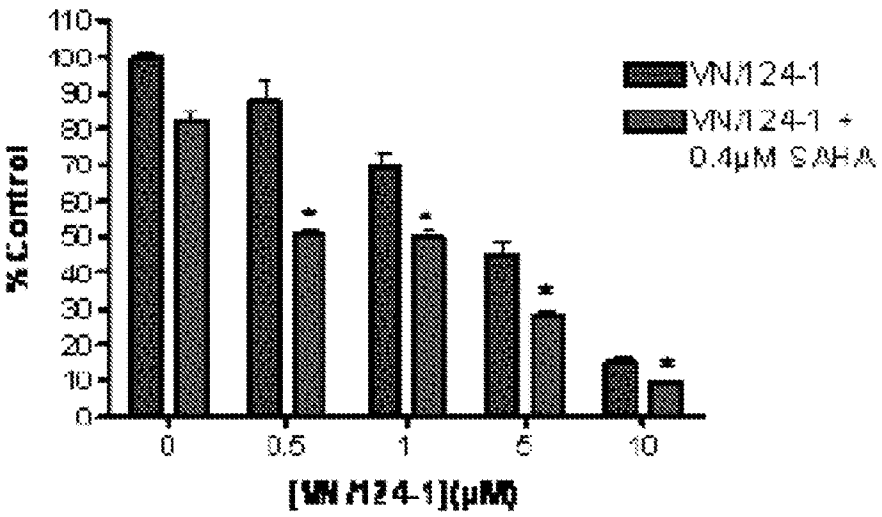

110
100
90
80
70
60
50
40
30
20
10
0
% Control
0
0.5
1
5
10
[VNP124-1](μM)
VNP124-1
VNP124-1 +
0.4μM SAHA
*
*
*
*

FIG. 9A

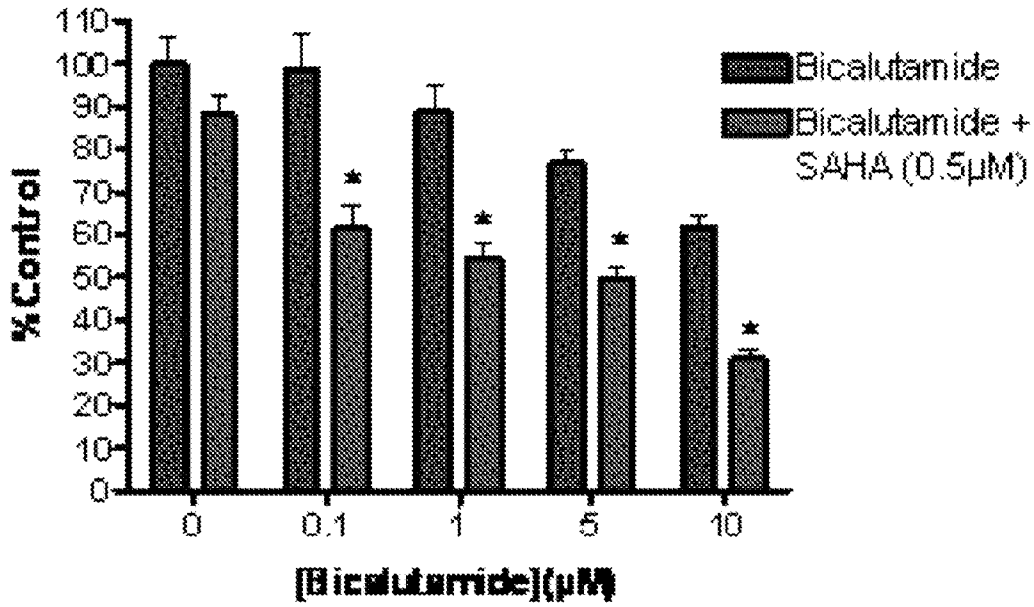

110
100
90
80
70
60
50
40
30
20
10
0
% Control
Bicalutamide
Bicalutamide + SAHA (0.5μM)
*
*
*
*
0
0.1
1
5
10
[Bicalutamide](μM)

FIG. 9B

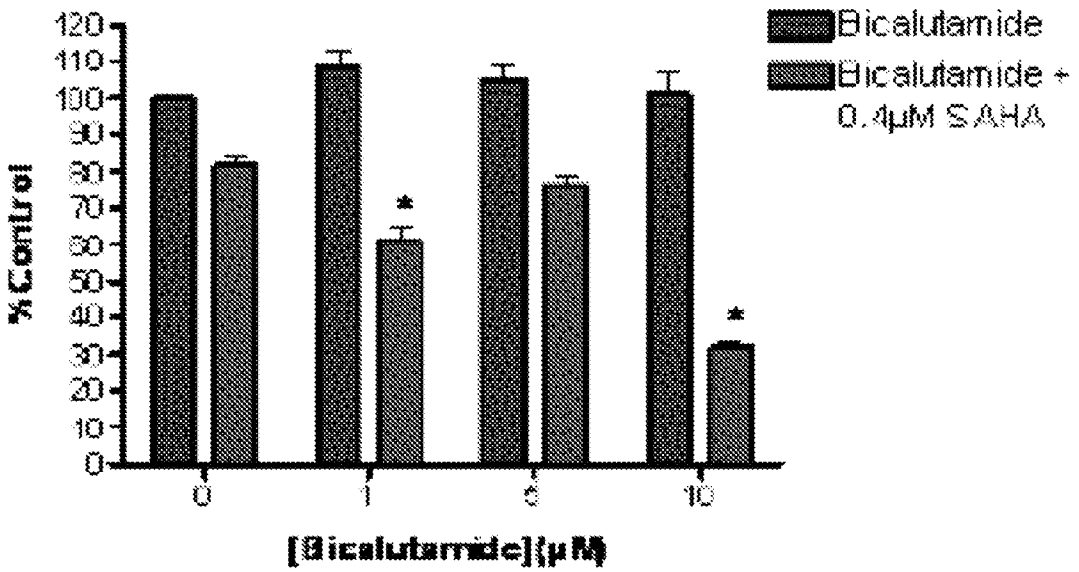
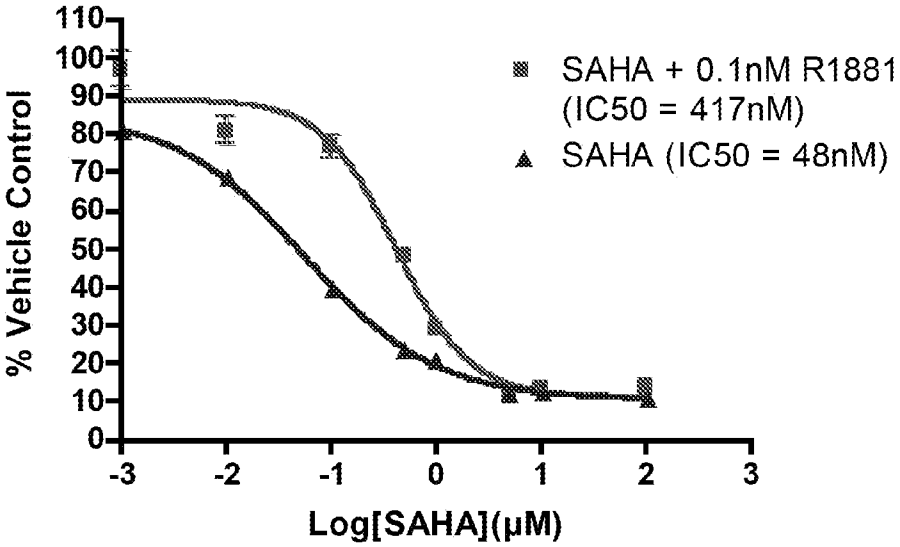

Bicalutamide
Bicalutamide + 0.4μM SAHA
% Control
120
110
100
90
80
70
60
50
40
30
20
10
0
*
*
0
1
5
10
[Bicalutamide](μM)

FIG. 10


% Vehicle Control
110
100
90
80
70
60
50
40
30
20
10
0
-3
-2
-1
0
1
2
3
Log[SAHA](μM)
SAHA + 0.1nM R1881
(IC50 = 417nM)
SAHA (IC50 = 48nM)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2008/066120

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/495(2006.01)i, A61K 31/44(2006.01)i, C07D 233/54(2006.01)i, C07C 259/10(2006.01)i, C07D 215/20(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 8 as above

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

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

STN, PubMed-online

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MARROCCO, D.L., ET AL., "SUBEROYLANILIDE HYDROXAMIC ACID(VORINOSTAT) REPRESSES ANDROGEN RECEPTOR ANTAGONIST TO INHIBIT PROSTATE CANCER CELL PROLIFERATION", MOLECULAR CANCER THERAPEUTICS, January 2007, Vol. 6, No. 1, pages 51-60. See abstract; fig. 7.	1-5, 16-19
Y	HANDRATTA, V.D. ET AL., "NOVEL C-17-HETEROARYL STEROIDAL CYP17 INHIBITORS/ANTIANDROGENS; SYNTHESIS, IN VITRO BIOLOGICAL ACTIVITY, PHARMACOKINETICS, AND ANTITUMOR ACTIVITY IN THE LAPC4 HUMAN PROSTATE CANCER XENOGRAFT MODEL", JOURNAL OF MEDICINAL CHEMISTRY, 2005, Vol. 48, No. 8, pages 2972-2984. See abstract; fig. 2.	1-5, 16-19
A	WO 2007/056243 A2 (MERCK & CO. INC.) 18 May 2007 See the whole document.	1-5, 16-19

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 OCTOBER 2008 (27.10.2008)	**27 OCTOBER 2008 (27.10.2008)**
Name and mailing address of the ISA/KR Korean Intellectual Property Office Government Complex-Daejeon, 139 Seonsa-ro, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer KANG, TAI HYUN Telephone No. 82-42-481-5627

Form PCT/ISA/210 (second sheet) (July 2008)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2008/066120

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-19
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 1-19 pertain to method for diagnosis and treatment of the human or animal body by therapy and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search. Although Claims 1-19 are directed to a method of diagnosis and treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

2. ☒ Claims Nos.: 8,9
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Since claims 8,9 refer to claim 7, which are not drafted in accordance with the third sentences of Rule 6.4(a).

3. ☒ Claims Nos.: 6, 7, 10-15
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2008/066120

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007/056243 A2	18.05.2007	WO 2007/056135 A1	18.05.2007
		WO 2007/056162 A2	18.05.2007
		US 20070117815 A1	24.05.2007
		EP 1954284 A1	13.08.2008
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