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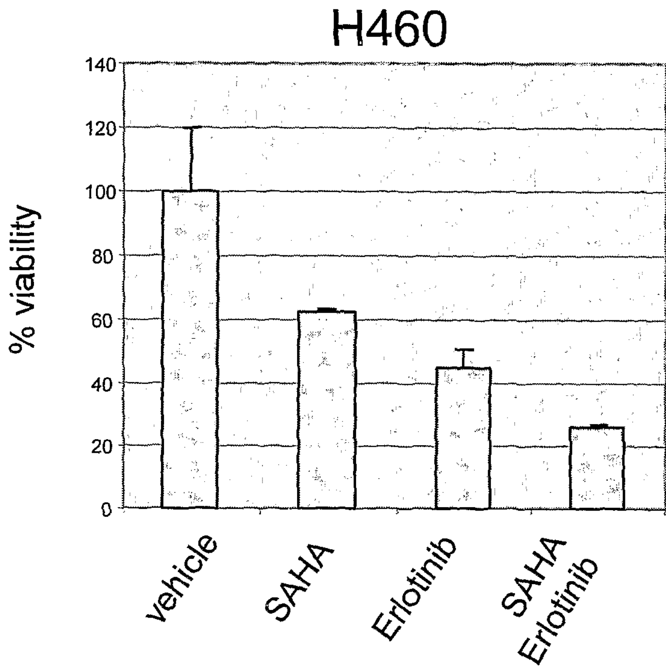

(57) Abstract: The present invention relates to a method of treating cancer in a subject in need thereof, by administering to a subject in need thereof a first amount of a histone deacetylase (HDAC) inhibitor such as suberoylanilide hydroxamic acid (SAHA), or a pharmaceutically acceptable salt or hydrate thereof, and a second amount of one or more anti-cancer agents, including Erlotinib. The HDAC inhibitor and the anti-cancer agent may be administered to comprise therapeutically effective amounts. In various aspects, the effect of the HDAC inhibitor and the anti-cancer agent may be additive or synergistic.

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METHODS OF USING SAHA AND ERLOTINIB FOR TREATING CANCER

5 ## CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority from U.S. Provisional Application Serial No. 60/733,666, filed on November 4, 2005.

Each of the applications and patents cited in this text, as well as each document or reference cited in each of the applications and patents (including during the prosecution of each 10 issued patent; "application cited documents"), and each of the U.S. and foreign applications or patents corresponding to and/or claiming priority from any of these applications and patents, and each of the documents cited or referenced in each of the application cited documents, are hereby expressly incorporated herein by reference. More generally, documents or references are cited in this text, either in a Reference List before the claims, or in the text itself; and, each of these 15 documents or references ("herein-cited references"), as well as each document or reference cited in each of the herein-cited references (including any manufacturer's specifications, instructions, etc.), is hereby expressly incorporated herein by reference. Documents incorporated by reference into this text may be employed in the practice of the invention.

20 ## FIELD OF THE INVENTION

The present invention relates to a method of treating cancer by administering a histone deacetylase (HDAC) inhibitor such as suberoylanilide hydroxamic acid (SAHA) in combination with one or more anti-cancer agents, including Erlotinib. The combined amounts together can comprise a therapeutically effective amount.

25

BACKGROUND OF THE INVENTION

Cancer is a disorder in which a population of cells has become, in varying degrees, unresponsive to the control mechanisms that normally govern proliferation and differentiation.

Therapeutic agents used in clinical cancer therapy can be categorized into several groups, 30 including, alkylating agents, antibiotic agents, antimetabolic agents, biologic agents, hormonal agents, and plant-derived agents.

Cancer therapy is also being attempted by the induction of terminal differentiation of the neoplastic cells (M. B., Roberts, A. B., and Driscoll, J. S. (1985) in *Cancer: Principles and Practice of Oncology*, eds. Hellman, S., Rosenberg, S. A., and DeVita, V. T., Jr., Ed. 2, (J. B. Lippincott, Philadelphia), P. 49). In cell culture models, differentiation has been reported by exposure of cells to a variety of stimuli, including: cyclic AMP and retinoic acid (Breitman, T. R., Selonick, S. E., and Collins, S. J. (1980) *Proc. Natl. Acad. Sci. USA* 77: 2936-2940; Olsson, I. L. and Breitman, T. R. (1982) *Cancer Res.* 42: 3924-3927), aclarubicin and other anthracyclines (Schwartz, E. L. and Sartorelli, A. C. (1982) *Cancer Res.* 42: 2651-2655). There is abundant evidence that neoplastic transformation does not necessarily destroy the potential of cancer cells to differentiate (Sporn *et al*; Marks, P. A., Sheffery, M., and Rifkind, R. A. (1987) *Cancer Res.* 47: 659; Sachs, L. (1978) *Nature (Lond.)* 274: 535).

There are many examples of tumor cells which do not respond to the normal regulators of proliferation and appear to be blocked in the expression of their differentiation program, and yet can be induced to differentiate and cease replicating. A variety of agents can induce various transformed cell lines and primary human tumor explants to express more differentiated characteristics. Histone deacetylase inhibitors such as suberoylanilide hydroxamide acid (SAHA), belong to this class of agents that have the ability to induce tumor cell growth arrest, differentiation, and/or apoptosis (Richon, V.M., Webb, Y., Merger, R., *et al.* (1996) *PNAS* 93:5705-8). These compounds are targeted towards mechanisms inherent to the ability of a neoplastic cell to become malignant, as they do not appear to have toxicity in doses effective for inhibition of tumor growth in animals (Cohen, L.A., Amin, S., Marks, P.A., Rifkind, R.A., Desai, D., and Richon, V.M. (1999) *Anticancer Research* 19:4999-5006). There are several lines of evidence that histone acetylation and deacetylation are mechanisms by which transcriptional regulation in a cell is achieved (Grunstein, M. (1997) *Nature* 389:349-52). These effects are thought to occur through changes in the structure of chromatin by altering the affinity of histone proteins for coiled DNA in the nucleosome.

There are five types of histones that have been identified (designated H1, H2A, H2B, H3 and H4). Histones H2A, H2B, H3, and H4 are found in the nucleosomes and H1 is a linker located between nucleosomes. Each nucleosome contains two of each histone type within its core, except for H1, which is present singly in the outer portion of the nucleosome structure. It is believed that when the histone proteins are hypoacetylated, there is a greater affinity of the

histone to the DNA phosphate backbone. This affinity causes DNA to be tightly bound to the histone and renders the DNA inaccessible to transcriptional regulatory elements and machinery. The regulation of acetylated states occurs through the balance of activity between two enzyme complexes, histone acetyl transferase (HAT) and histone deacetylase (HDAC). The
5 hypoacetylated state is thought to inhibit transcription of associated DNA. This hypoacetylated state is catalyzed by large multiprotein complexes that include HDAC enzymes. In particular, HDACs have been shown to catalyze the removal of acetyl groups from the chromatin core histones.

The epidermal growth factor receptor (EGFR) is part of a subfamily of four closely
10 related receptor tyrosine kinases including EGFR (ErbB-1; HER-1), HER-2/neu (ErbB-2), HER-3 (ErbB-3), and HER-4 (ErbB-4) (see, e.g., Sedlacek, *Drugs*, 59: 435-476, 2000; Wells A., *Int. J. Biochem. Cell Biol.*, 31: 637-643, 1999; Ciardiello and Tortora, *Clin. Cancer Res.* 7:2958-2970, 2001; Hynes and Lane, *Nat. Rev. Cancer* 5(5):341-354, 2005). All ErbB members have an extracellular ligand-binding region, a single membrane-spanning region, and a cytoplasmic
15 tyrosine-kinase domain. The receptors are expressed in epithelial, mesenchymal, neuronal, and other tissues. Under physiological conditions, activation of the ErbB receptors is controlled by the spatial and temporal expression of their ligands, which are members of the EGF family of growth factors (see, e.g., Riese and Stern, *Bioessays* 20:41-48, 1998; Yarden and Silwkowski, *Nature Rev. Mol. Cell Biol.* 2:127-137, 2001).

20 Ligand binding to ErbB receptors induces the formation of receptor homo- and heterodimers and activation of the receptor kinase domain. This results in phosphorylation on specific tyrosine residues within the cytoplasmic tail. The phosphorylated residues serve as docking sites for a range of proteins, the recruitment of which leads to the activation of intracellular signaling pathways (see, e.g., Yarden and Silwkowski, *Nature Rev. Mol. Cell Biol.*
25 2:127-137, 2001; Olayioye *et al.*, *EMBO J.* 19:3159-3167, 2000; Schlessinger, *Science* 306:1506-1507, 2004; Hynes and Lane, *Nat. Rev. Cancer* 5(5):341-354, 2005). The signaling cascade leads to activation of ras and mitogen-activated protein kinase, which in turn activate several nuclear proteins, including cyclin D1, a protein required for cell cycle progression from G1 to S phase (Wells A., *Int. J. Biochem. Cell Biol.*, 31: 637-643, 1999; Perry J. E. *et al.*,
30 *Prostate*, 35: 117-124, 1998). Inhibitors of ErbB receptor tyrosine kinases, particularly EGFR inhibitors, are among the most intensely studied new molecular therapeutic agents. Erlotinib

(e.g., Tarceva™, OSI Pharmaceuticals, Inc.) is an orally active, potent, selective inhibitor of the EGFR tyrosine kinase.

Besides the aim to increase the therapeutic efficacy, another purpose of combination treatment is the potential decrease of the doses of the individual components in the resulting combinations in order to decrease unwanted or harmful side effects caused by higher doses of the individual components. Thus, there is an urgent need to discover suitable methods for the treatment of cancer, including combination treatments that result in decreased side effects and that are effective at treating and controlling malignancies.

SUMMARY OF THE INVENTION

The present invention is based on the discovery that histone deacetylase (HDAC) inhibitors, for example suberoylanilide hydroxamic acid (SAHA), can be used in combination with Erlotinib, to provide therapeutic efficacy.

The invention relates to a method for treating cancer or other disease comprising administering to a subject in need thereof an amount of an HDAC inhibitor, e.g., SAHA, an amount of a second anti-cancer agent, e.g., Erlotinib, and optionally an amount of a third anti-cancer agent.

The invention further relates to pharmaceutical combinations useful for the treatment of cancer or other disease comprising an amount of an HDAC inhibitor, e.g., SAHA, an amount of a second anti-cancer agent, e.g., Erlotinib, and optionally an amount of a third anti-cancer agent.

The invention further relates to the use of an amount of an HDAC inhibitor, e.g., SAHA, an amount of a second anti-cancer agent, e.g., Erlotinib, and optionally an amount of a third anti-cancer agent, for the manufacture of one or more medicaments for treating cancer or other disease.

The invention further relates to methods for selectively inducing terminal differentiation, cell growth arrest, and/or apoptosis of neoplastic cells, thereby inhibiting proliferation of such cells in a subject by administering to the subject an amount of an HDAC inhibitor, e.g., SAHA, an amount of a second anti-cancer agent, e.g., Erlotinib, and optionally an amount of a third anti-cancer agent, wherein the HDAC inhibitor and the one or more anti-cancer agents are administered in amounts effective to induce terminal differentiation, cell growth arrest, or apoptosis of the cells.

The invention further relates to *in vitro* methods for selectively inducing terminal differentiation, cell growth arrest, and/or apoptosis of neoplastic cells, thereby inhibiting proliferation of such cells, by contacting the cells with an amount of an HDAC inhibitor, e.g., SAHA, an amount of a second anti-cancer agent, e.g., Erlotinib, and optionally an amount of a third anti-cancer agent, wherein the HDAC inhibitor and the one or more anti-cancer agents are administered in amounts effective to induce terminal differentiation, cell growth arrest, or apoptosis of the cells.

In the context of the present invention, the combined treatments together can comprise a therapeutically effective amount. In addition, the combination of the HDAC inhibitor and one or more anti-cancer agents can provide additive or synergistic therapeutic effects.

HDAC inhibitors suitable for use in the present invention include but are not limited to hydroxamic acid derivatives, such as SAHA, Short Chain Fatty Acids (SCFAs), cyclic tetrapeptides, benzamide derivatives, or electrophilic ketone derivatives.

The treatment procedures described herein can be performed sequentially in any order, alternating in any order, simultaneously, or any combination thereof. In particular, the administration of an HDAC inhibitor, e.g., SAHA, the administration of the second anti-cancer agent, e.g., Erlotinib, and optionally, the administration of the third anti-cancer agent can be performed concurrently, consecutively, or, for example, alternating concurrent and consecutive administration.

The HDAC inhibitor can be administered in combination with a tyrosine kinase inhibitor, e.g., Erlotinib, and optionally in combination with any one or more of an additional HDAC inhibitor, an alkylating agent, an antibiotic agent, an antimetabolic agent, a hormonal agent, a plant-derived agent, an anti-angiogenic agent, a differentiation inducing agent, a cell growth arrest inducing agent, an apoptosis inducing agent, a cytotoxic agent, a biologic agent, a gene therapy agent, a retinoid agent, or an additional tyrosine kinase inhibitor.

According to the invention, the HDAC inhibitor is SAHA, which can be administered in combination with a tyrosine kinase inhibitor Erlotinib, and optionally in combination with any one or more of another HDAC inhibitor, an alkylating agent, an antibiotic agent, an antimetabolic agent, a hormonal agent, a plant-derived agent, an anti-angiogenic agent, a differentiation inducing agent, a cell growth arrest inducing agent, an apoptosis inducing agent, a cytotoxic

agent, a biologic agent, a gene therapy agent, a retinoid agent, or another tyrosine kinase inhibitor.

The combination therapy of the present invention can be used to treat inflammatory diseases, autoimmune diseases, allergic diseases, diseases associated with oxidative stress, neurodegenerative diseases, and diseases characterized by cellular hyperproliferation(e.g., cancers), or any combination thereof. In particular, the combination therapy can be used to treat diseases such as leukemia, lymphoma, myeloma, sarcoma, carcinoma, solid tumors, or any combination thereof.

In such combination therapies, SAHA can be administered in combination with a tyrosine kinase inhibitor such as Erlotinib. In particular embodiments, SAHA and Erlotinib are administered in combination for use in the treatment of lung cancer. In other particular embodiments, SAHA and Erlotinib are administered in combination for use in treating non-small cell lung cancer (NSCLC).

Accordingly, in one aspect of the present invention, a method of treating cancer in a subject in need thereof is provided, comprising administering to the subject a histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA), represented by the structure:

or a pharmaceutically acceptable salt or hydrate thereof, and a tyrosine kinase inhibitor, Erlotinib, represented by the structure:

or a pharmaceutically acceptable salt or hydrate thereof, wherein the histone deacetylase inhibitor and the tyrosine kinase inhibitor are administered in amounts effective for treating the cancer.

In one embodiment, the histone deacetylase inhibitor and the tyrosine kinase inhibitor are administered concurrently. In another embodiment, the histone deacetylase inhibitor is

administered prior to administering the tyrosine kinase inhibitor. In other embodiments, the histone deacetylase inhibitor is administered after administering the tyrosine kinase inhibitor. The histone deacetylase inhibitor and the tyrosine kinase inhibitor can be administered orally. Preferably, in the methods of the present invention, suberoylanilide hydroxamic acid (SAHA) and Erlotinib are administered. The cancer can be, for example, non-small cell lung cancer.

Another embodiment of the invention provides that the histone deacetylase inhibitor is administered once daily at a dose of 300 mg, wherein the administration is continuous. Alternatively, in another embodiment, the histone deacetylase inhibitor is administered once daily at a dose of 200 mg, 300 mg, 400 mg, or 500 mg for at least one period of 3 out of 7 days.

In other embodiments, the histone deacetylase inhibitor is administered twice daily at 200 mg or 300 mg per dose for at least one period of 3 out of 7 days. In some embodiments, the histone deacetylase inhibitor is administered for at least one period of 3 out of 7 days for two weeks, followed by a two-week rest period. In other embodiments, the histone deacetylase inhibitor is administered for at least one period of 3 out of 7 days for three weeks, followed by a one-week rest period. In yet other embodiments, the histone deacetylase inhibitor is administered for at least one period of 3 out of 7 days for one week, followed by a one-week rest period.

Another embodiment of the present invention provides that the histone deacetylase inhibitor is administered twice daily at 300 mg per dose for at least one period of 7 out of 14 days. In another embodiment, the histone deacetylase inhibitor is administered once daily at 300 mg per dose for at least one period of 14 out of 28 days.

The tyrosine kinase inhibitor can be administered once daily at a dose of 50 mg, 100 mg, or 150 mg, wherein the administration is continuous. In one embodiment, the histone deacetylase inhibitor is administered at a total daily dose of up to 400 mg and the tyrosine kinase inhibitor is administered at a total daily dose of up to 150 mg. Alternatively, the histone deacetylase inhibitor is administered at a total daily dose of up to 600 mg and the tyrosine kinase inhibitor is administered at a total daily dose of up to 150 mg.

Another aspect of the present invention provides an oral pharmaceutical composition comprising a histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA), represented by the structure:

or a pharmaceutically acceptable salt or hydrate thereof, and a tyrosine kinase inhibitor, Erlotinib, represented by the structure:

5 or a pharmaceutically acceptable salt or hydrate thereof, and optionally one or more pharmaceutically acceptable excipients. In one embodiment, the pharmaceutical composition comprises about 100 mg of SAHA and about 50 mg of Erlotinib. The pharmaceutical composition preferably comprises suberoylanilide hydroxamic acid (SAHA) and Erlotinib.

Unless otherwise defined, all technical and scientific terms used herein have the same
10 meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety. In cases of conflict, the present
15 specification, including definitions, will control. In addition, the materials, methods, and examples described herein are illustrative only and are not intended to be limiting.

Other features and advantages of the invention will be apparent from and are encompassed by the following detailed description and claims.

20 **BRIEF DESCRIPTION OF THE DRAWINGS**

The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of various embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale,
25 emphasis instead being placed upon illustrating the principles of the invention.

FIGS. 1A-1B: Cell viability was determined for non-small cell lung cancer cell lines H460 and A549 treated for 72 hours with the indicated concentrations of SAHA and Erlotinib alone and in combination. Percent viability was determined using the Vialight Assay (see Example 7). FIG. 1A: Results for H460 cells. FIG. 1B: Results for A549 cells.

5

DETAILED DESCRIPTION OF THE INVENTION

It has been unexpectedly discovered that a combination treatment procedure that includes administration of an HDAC inhibitor, SAHA, as described herein, and a tyrosine kinase inhibitor Erlotinib, as described herein, can provide improved therapeutic effects. Each of the treatments 10 (administration of an HDAC inhibitor, administration of the Erlotinib, and optionally, administration of a third-anti-cancer agent) is used to provide a therapeutically effective treatment.

The invention further relates to a method of treating cancer or other disease, in a subject in need thereof, by administering to a subject in need thereof an amount of suberoylanilide 15 hydroxamic acid (SAHA) or a pharmaceutically acceptable salt or hydrate thereof, in a treatment procedure, an amount of a tyrosine kinase inhibitor, such as Erlotinib, in another treatment procedure, and optionally an amount of a third anti-cancer agent in another treatment procedure, wherein the amounts can comprise a therapeutically effective amount. The effect of SAHA, the Erlotinib, and optional additional anti-cancer agent can be, e.g., additive or synergistic.

20 In one aspect, the method comprises administering to a patient in need thereof a first amount of SAHA or a pharmaceutically acceptable salt or hydrate thereof, in a first treatment procedure, a second amount of Erlotinib or a pharmaceutically acceptable salt or hydrate thereof, in a second treatment procedure, and optionally a third amount of an additional anti-cancer agent or a pharmaceutically acceptable salt or hydrate thereof, in a third treatment procedure. The 25 invention further relates to pharmaceutical combinations useful for the treatment of cancer or other disease. In one aspect, the pharmaceutical combination comprises a first amount of an HDAC inhibitor, e.g., SAHA or a pharmaceutically acceptable salt or hydrate thereof, a second amount of an anti-cancer agent, such as a tyrosine kinase inhibitor like Erlotinib or a pharmaceutically acceptable salt or hydrate thereof, and optionally a third amount of an 30 additional anti-cancer agent or a pharmaceutically acceptable salt or hydrate thereof. The first, second, and optional third amounts can comprise a therapeutically effective amount.

The combination therapy of the invention provides a therapeutic advantage in view of the differential toxicity associated with the two or more treatment modalities. For example, treatment with HDAC inhibitors can lead to a particular toxicity that is not seen with the one or more anti-cancer agents, and vice versa. As such, this differential toxicity can permit each treatment to be administered at a dose at which said toxicities do not exist or are minimal, such that together the combination therapy provides a therapeutic dose while avoiding the toxicities of each of the constituents of the combination agents. Furthermore, when the therapeutic effects achieved as a result of the combination treatment are enhanced or synergistic, for example, significantly better than additive therapeutic effects, the doses of each of the agents can be reduced even further, thus lowering the associated toxicities to an even greater extent.

Definitions

The term "treating" in its various grammatical forms in relation to the present invention refers to preventing (i.e. chemoprevention), curing, reversing, attenuating, alleviating, minimizing, suppressing or halting the deleterious effects of a disease state, disease progression, disease causative agent (e.g., bacteria or viruses) or other abnormal condition. For example, treatment may involve alleviating a symptom (i.e., not necessarily all symptoms) of a disease or attenuating the progression of a disease. Because some of the inventive methods involve the physical removal of the etiological agent, the artisan will recognize that they are equally effective in situations where the inventive compound is administered prior to, or simultaneous with, exposure to the etiological agent (prophylactic treatment) and situations where the inventive compounds are administered after (even well after) exposure to the etiological agent.

Treatment of cancer, as used herein, refers to partially or totally inhibiting, delaying or preventing the progression of cancer including cancer metastasis; inhibiting, delaying or preventing the recurrence of cancer including cancer metastasis; or preventing the onset or development of cancer (chemoprevention) in a mammal, for example a human. In addition, the method of the present invention is intended for the treatment of chemoprevention of human patients with cancer. However, it is also likely that the method would be effective in the treatment of cancer in other mammals.

The "anti-cancer agents" of the invention encompass those described herein, including any pharmaceutically acceptable salts or hydrates of such agents, or any free acids, free bases, or

other free forms of such agents, and as non-limiting examples: A) Polar compounds (Marks *et al.* (1987); Friend, C., Scher, W., Holland, J. W., and Sato, T. (1971) *Proc. Natl. Acad. Sci. (USA)* 68: 378-382; Tanaka, M., Levy, J., Terada, M., Breslow, R., Rifkind, R. A., and Marks, P. A. (1975) *Proc. Natl. Acad. Sci. (USA)* 72: 1003-1006; Reuben, R. C., Wife, R. L., Breslow,
5 R., Rifkind, R. A., and Marks, P. A. (1976) *Proc. Natl. Acad. Sci. (USA)* 73: 862-866); B) Derivatives of vitamin D and retinoic acid (Abe, E., Miyaura, C., Sakagami, H., Takeda, M., Konno, K., Yamazaki, T., Yoshika, S., and Suda, T. (1981) *Proc. Natl. Acad. Sci. (USA)* 78: 4990-4994; Schwartz, E. L., Snoddy, J. R., Kreutter, D., Rasmussen, H., and Sartorelli, A. C. (1983) *Proc. Am. Assoc. Cancer Res.* 24: 18; Tanenaga, K., Hozumi, M., and Sakagami, Y.
10 (1980) *Cancer Res.* 40: 914-919); C) Steroid hormones (Lotem, J. and Sachs, L. (1975) *Int. J. Cancer 15:* 731-740); D) Growth factors (Sachs, L. (1978) *Nature (Lond.)* 274: 535, Metcalf, D. (1985) *Science*, 229: 16-22); E) Proteases (Scher, W., Scher, B. M., and Waxman, S. (1983) *Exp. Hematol.* 11: 490-498; Scher, W., Scher, B. M., and Waxman, S. (1982) *Biochem. & Biophys. Res. Comm.* 109: 348-354); F) Tumor promoters (Huberman, E. and Callaham, M. F.
15 (1979) *Proc. Natl. Acad. Sci. (USA)* 76: 1293-1297; Lottem, J. and Sachs, L. (1979) *Proc. Natl. Acad. Sci. (USA)* 76: 5158-5162); and G) Inhibitors of DNA or RNA synthesis (Schwartz, E. L. and Sartorelli, A. C. (1982) *Cancer Res. 42:* 2651-2655, Terada, M., Epner, E., Nudel, U., Salmon, J., Fibach, E., Rifkind, R. A., and Marks, P. A. (1978) *Proc. Natl. Acad. Sci. (USA)* 75: 2795-2799; Morin, M. J. and Sartorelli, A. C. (1984) *Cancer Res. 44*: 2807-2812; Schwartz, E.
20 L., Brown, B. J., Nierenberg, M., Marsh, J. C., and Sartorelli, A. C. (1983) *Cancer Res.* 43: 2725-2730; Sugano, H., Furusawa, M., Kawaguchi, T., and Ikawa, Y. (1973) *Bibl. Hematol.* 39: 943-954; Ebert, P. S., Wars, I., and Buell, D. N. (1976) *Cancer Res.* 36: 1809-1813; Hayashi, M., Okabe, J., and Hozumi, M. (1979) *Gann* 70: 235-238).

As used herein, the term "therapeutically effective amount" is intended to qualify the
25 combined amount of treatments in the combination therapy. The combined amount will achieve the desired biological response. In the present invention, the desired biological response is partial or total inhibition, delay or prevention of the progression of cancer including cancer metastasis; inhibition, delay or prevention of the recurrence of cancer including cancer metastasis; or the prevention of the onset or development of cancer (chemoprevention) in a
30 mammal, for example a human.

As used herein, the terms "combination treatment", "combination therapy", "combined treatment," or "combinatorial treatment", used interchangeably, refer to a treatment of an individual with at least two different therapeutic agents. According to one aspect of the invention, the individual is treated with a first therapeutic agent, e.g., SAHA or another HDAC
5 inhibitor as described herein. The second therapeutic agent may be another HDAC inhibitor, or may be any clinically established anti-cancer agent (such as a tyrosine kinase inhibitor like Erlotinib) as defined herein. A combinatorial treatment may include a third or even further therapeutic agent. The combination treatments may be carried out consecutively or concurrently.

A "retinoid" or "retinoid agent" (e.g., 3-methyl TTNEB) as used herein encompasses any
10 synthetic, recombinant, or naturally-occurring compound that binds to one or more retinoid receptors, including any pharmaceutically acceptable salts or hydrates of such agents, and any free acids, free bases, or other free forms of such agents.

A "tyrosine kinase inhibitor" (e.g., Erlotinib) encompasses any synthetic, recombinant, or naturally occurring agent that binds to or otherwise decreases the activity or levels of one or
15 more tyrosine kinases (e.g., receptor tyrosine kinases), including any pharmaceutically acceptable salts or hydrates of such inhibitors, and any free acids, free bases, or other free forms of such inhibitors. Included are tyrosine kinase inhibitors that act on EGFR (ErbB-1; HER-1). Also included are tyrosine kinase inhibitors that act specifically on EGFR. Non-limiting examples of tyrosine kinases inhibitors are provided herein.

20 As recited herein, "HDAC inhibitor" (e.g., SAHA) encompasses any synthetic, recombinant, or naturally-occurring inhibitor, including any pharmaceutical salts or hydrates of such inhibitors, and any free acids, free bases, or other free forms of such inhibitors. "Hydroxamic acid derivative," as used herein, refers to the class of histone deacetylase inhibitors that are hydroxamic acid derivatives. Specific examples of inhibitors are provided herein.

25 "Patient" or "subject" as the terms are used herein, refer to the recipient of the treatment. Mammalian and non-mammalian patients are included. In a specific embodiment, the patient is a mammal, such as a human, canine, murine, feline, bovine, ovine, swine, or caprine. In a particular embodiment, the patient is a human.

The terms "intermittent" or "intermittently" as used herein means stopping and starting at
30 either regular or irregular intervals.

The term "hydrate" includes but is not limited to hemihydrate, monohydrate, dihydrate, trihydrate, and the like.

Histone Deacetylases and Histone Deacetylase Inhibitors

Histone deacetylases (HDACs) include enzymes that catalyze the removal of acetyl groups from lysine residues in the amino terminal tails of the nucleosomal core histones. As such, HDACs together with histone acetyl transferases (HATs) regulate the acetylation status of histones. Histone acetylation affects gene expression and inhibitors of HDACs, such as the hydroxamic acid-based hybrid polar compound suberoylanilide hydroxamic acid (SAHA) induce growth arrest, differentiation, and/or apoptosis of transformed cells *in vitro* and inhibit tumor growth *in vivo*.

HDACs can be divided into three classes based on structural homology. Class I HDACs (HDACs 1, 2, 3, and 8) bear similarity to the yeast RPD3 protein, are located in the nucleus and are found in complexes associated with transcriptional co-repressors. Class II HDACs (HDACs 4, 5, 6, 7 and 9) are similar to the yeast HDA1 protein, and have both nuclear and cytoplasmic subcellular localization. Both Class I and II HDACs are inhibited by hydroxamic acid-based HDAC inhibitors, such as SAHA. Class III HDACs form a structurally distant class of NAD dependent enzymes that are related to the yeast SIR2 proteins and are not inhibited by hydroxamic acid-based HDAC inhibitors.

Histone deacetylase inhibitors or HDAC inhibitors are compounds that are capable of inhibiting the deacetylation of histones *in vivo*, *in vitro* or both. As such, HDAC inhibitors inhibit the activity of at least one histone deacetylase. As a result of inhibiting the deacetylation of at least one histone, an increase in acetylated histone occurs and accumulation of acetylated histone is a suitable biological marker for assessing the activity of HDAC inhibitors. Therefore, procedures that can assay for the accumulation of acetylated histones can be used to determine the HDAC inhibitory activity of compounds of interest. It is understood that compounds that can inhibit histone deacetylase activity can also bind to other substrates and as such can inhibit other biologically active molecules such as enzymes. It is also to be understood that the compounds of the present invention are capable of inhibiting any of the histone deacetylases set forth above, or any other histone deacetylases.

For example, in patients receiving HDAC inhibitors, the accumulation of acetylated histones in peripheral mononuclear cells as well as in tissue treated with HDAC inhibitors can be determined against a suitable control.

HDAC inhibitory activity of a particular compound can be determined *in vitro* using, for 5 example, an enzymatic assay which shows inhibition of at least one histone deacetylase. Further, determination of the accumulation of acetylated histones in cells treated with a particular composition can be determinative of the HDAC inhibitory activity of a compound.

Assays for the accumulation of acetylated histones are well known in the literature. See, for example, Marks, P.A. *et al.*, *J. Natl. Cancer Inst.*, 92:1210-1215, 2000, Butler, L.M. *et al.*, 10 *Cancer Res.* 60:5165-5170 (2000), Richon, V. M. *et al.*, *Proc. Natl. Acad. Sci., USA*, 95:3003-3007, 1998, and Yoshida, M. *et al.*, *J. Biol. Chem.*, 265:17174-17179, 1990.

For example, an enzymatic assay to determine the activity of an HDAC inhibitor compound can be conducted as follows. Briefly, the effect of an HDAC inhibitor compound on affinity purified human epitope-tagged (Flag) HDAC1 can be assayed by incubating the enzyme 15 preparation in the absence of substrate on ice for about 20 minutes with the indicated amount of inhibitor compound. Substrate ([^{3}H]acetyl-labeled murine erythroleukemia cell-derived histone) can be added and the sample can be incubated for 20 minutes at 37°C in a total volume of 30 μL. The reaction can then be stopped and released acetate can be extracted and the amount of radioactivity release determined by scintillation counting. An alternative assay useful for 20 determining the activity of an HDAC inhibitor compound is the "HDAC Fluorescent Activity Assay; Drug Discovery Kit-AK-500" available from BIOMOL® Research Laboratories, Inc., Plymouth Meeting, PA.

In vivo studies can be conducted as follows. Animals, for example, mice, can be injected intraperitoneally with an HDAC inhibitor compound. Selected tissues, for example, brain, 25 spleen, liver etc, can be isolated at predetermined times, post administration. Histones can be isolated from tissues essentially as described by Yoshida *et al.*, *J. Biol. Chem.* 265:17174-17179, 1990. Equal amounts of histones (about 1 μg) can be electrophoresed on 15% SDS-polyacrylamide gels and can be transferred to Hybond-P filters (available from Amersham). Filters can be blocked with 3% milk and can be probed with a rabbit purified polyclonal anti-30 acetylated histone H4 antibody (αAc-H4) and anti-acetylated histone H3 antibody (αAc-H3) (Upstate Biotechnology, Inc.). Levels of acetylated histone can be visualized using a horseradish

peroxidase-conjugated goat anti-rabbit antibody (1:5000) and the SuperSignal chemiluminescent substrate (Pierce). As a loading control for the histone protein, parallel gels can be run and stained with Coomassie Blue (CB).

In addition, hydroxamic acid-based HDAC inhibitors have been shown to up regulate the expression of the $p21_{WAF1}$ gene. The $p21_{WAF1}$ protein is induced within 2 hours of culture with HDAC inhibitors in a variety of transformed cells using standard methods. The induction of the $p21_{WAF1}$ gene is associated with accumulation of acetylated histones in the chromatin region of this gene. Induction of $p21_{WAF1}$ can therefore be recognized as involved in the G1 cell cycle arrest caused by HDAC inhibitors in transformed cells.

U.S. Patent Numbers 5,369,108, 5,932,616, 5,700,811, 6,087,367 and 6,511,990, issued to some of the present inventors, disclose compounds useful for selectively inducing terminal differentiation of neoplastic cells, which compounds have two polar end groups separated by a flexible chain of methylene groups or a by a rigid phenyl group, wherein one or both of the polar end groups is a large hydrophobic group. Some of the compounds have an additional large hydrophobic group at the same end of the molecule as the first hydrophobic group which further increases differentiation activity about 100 fold in an enzymatic assay and about 50 fold in a cell differentiation assay. Methods of synthesizing the compounds used in the methods and pharmaceutical compositions of this invention are fully described the aforementioned patents, the entire contents of which are incorporated herein by reference.

Thus, the present invention includes within its broad scope compositions comprising HDAC inhibitors which are 1) hydroxamic acid derivatives; 2) Short-Chain Fatty Acids (SCFAs); 3) cyclic tetrapeptides; 4) benzamides; 5) electrophilic ketones; and/or any other class of compounds capable of inhibiting histone deacetylases, for use in inhibiting histone deacetylase, inducing terminal differentiation, cell growth arrest and/or apoptosis in neoplastic cells, and/or inducing differentiation, cell growth arrest and/or apoptosis of tumor cells in a tumor.

Non-limiting examples of such HDAC inhibitors are set forth below. It is understood that the present invention includes any salts, crystal structures, amorphous structures, hydrates, derivatives, metabolites, stereoisomers, structural isomers, and prodrugs of the HDAC inhibitors described herein.

A. **Hydroxamic Acid Derivatives** such as Suberoylanilide hydroxamic acid (SAHA)

(Richon *et al.*, *Proc. Natl. Acad. Sci. USA* 95,3003-3007 (1998)); m-Carboxycinnamic acid bishydroxamide (CBHA) (Richon *et al.*, *supra*); Pyroxamide; Trichostatin analogues such as Trichostatin A (TSA) and Trichostatin C (Koghe *et al.* 1998. *Biochem. Pharmacol.* 56: 1359-1364); Salicylbishydroxamic acid (Andrews *et al.*, *International J. Parasitology* 30,761-768
5 (2000)); Suberoyl bishydroxamic acid (SBHA) (U.S. Patent 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 (3Cl-UCHA); Oxamflatin [(2E)-5-[3-[(phenylsufonyl) aminol phenyl]-pent-2-en-4-ynohydroxamic acid] (Kim *et al.* *Oncogene*, 18: 2461 2470 (1999)); A-161906, Scriptaid (Su *et*
10 *al.* 2000 *Cancer Research*, 60: 3137-3142); PXD-101 (Prolifix); LAQ-824; CHAP; MW2796 (Andrews *et al.*, *supra*); MW2996 (Andrews *et al.*, *supra*); or any of the hydroxamic acids disclosed in U.S. Patent Numbers 5,369,108, 5,932,616, 5,700,811, 6,087,367, and 6,511,990.

B. **Cyclic Tetrapeptides** such as Trapoxin A (TPX)-cyclic tetrapeptide (cyclo-(L-phenylalanyl-L-phenylalanyl-D-pipecolinyl-L-2-amino-8-oxo-9,10-epoxy decanoyl)) (Kijima *et*
15 *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 February 2000)); 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* 93,13143-13147 (1996)); Apicidin Ia, Apicidin Ib, Apicidin Ic,
20 Apicidin IIa, and Apicidin IIb (P. Dulski *et al.*, PCT Application WO 97/11366); CHAP, HC-toxin cyclic tetrapeptide (Bosch *et al.*, *Plant Cell* 7, 1941-1950 (1995)); WF27082 cyclic tetrapeptide (PCT Application WO 98/48825); and Chlamydocin (Bosch *et al.*, *supra*).

C. **Short chain fatty acid (SCFA) derivatives** such as: Sodium Butyrate (Cousens *et al.*, *J. Biol. Chem.* 254,1716-1723 (1979)); Isovalerate (McBain *et al.*, *Biochem. Pharm.* 53:
25 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*,
30 60,749-755 (2000)); Valproic acid, Valproate, and Pivanex™.

D. **Benzamide derivatives** such as CI-994; MS-275 [N- (2-aminophenyl)-4-[N-

(pyridin-3-yl methoxycarbonyl) aminomethyl] benzamide] (Saito *et al., Proc. Natl. Acad. Sci. USA* 96, 4592-4597 (1999)); and 3'-amino derivative of MS-275 (Saito *et al., supra*).

E. **Electrophilic ketone derivatives** such as Trifluoromethyl ketones (Frey *et al, Bioorganic & Med. Chem. Lett.* (2002), 12, 3443-3447; U.S. 6,511,990) and α-keto amides such as N-methyl- α-ketoamides.

F. **Other HDAC Inhibitors** such as natural products, psammaplins, and Depudecin (Kwon *et al.* 1998. PNAS 95: 3356-3361).

Hydroxamic acid based HDAC inhibitors include suberoylanilide hydroxamic acid (SAHA), m-carboxycinnamic acid bishydroxamate (CBHA) and pyroxamide. SAHA has been shown to bind directly in the catalytic pocket of the histone deacetylase enzyme. SAHA induces cell cycle arrest, differentiation, and/or apoptosis of transformed cells in culture and inhibits tumor growth in rodents. SAHA is effective at inducing these effects in both solid tumors and hematological cancers. It has been shown that SAHA is effective at inhibiting tumor growth in animals with no toxicity to the animal. The SAHA-induced inhibition of tumor growth is associated with an accumulation of acetylated histones in the tumor. SAHA is effective at inhibiting the development and continued growth of carcinogen-induced (N-methylnitrosourea) mammary tumors in rats. SAHA was administered to the rats in their diet over the 130 days of the study. Thus, SAHA is a nontoxic, orally active antitumor agent whose mechanism of action involves the inhibition of histone deacetylase activity.

HDAC inhibitors include those disclosed in U.S. Patent Numbers 5,369,108, 5,932,616, 5,700,811, 6,087,367, and 6,511,990, issued to some of the present inventors disclose compounds, the entire contents of which are incorporated herein by reference, non-limiting examples of which are set forth below:

Specific HDAC inhibitors include suberoylanilide hydroxamic acid (SAHA; *N*-Hydroxy-*N′*-phenyl octanediamide), which is represented by the following structural formula:

Other examples of such compounds and other HDAC inhibitors can be found in U.S. Patent No. 5,369,108, issued on November 29, 1994, U.S. Patent No. 5,700,811, issued on December 23, 1997, U.S. Patent No. 5,773,474, issued on June 30, 1998, U.S. Patent No.

5,932,616, issued on August 3, 1999 and U.S. Patent No. 6,511,990, issued January 28, 2003, all to Breslow *et al.*; U.S. Patent No. 5,055,608, issued on October 8, 1991, U.S. Patent No. 5,175,191, issued on December 29, 1992 and U.S. Patent No. 5,608,108, issued on March 4, 1997, all to Marks *et al.*; as well as Yoshida, M., *et al.*, *Bioassays* 17, 423-430 (1995); Saito, A., *et al.*, *PNAS USA* 96, 4592-4597, (1999); Furamai R. *et al.*, *PNAS USA* 98 (1), 87-92 (2001); Komatsu, Y., *et al.*, *Cancer Res.* 61(11), 4459-4466 (2001); Su, G.H., *et al.*, *Cancer Res.* 60, 3137-3142 (2000); Lee, B.I. *et al.*, *Cancer Res.* 61(3), 931-934; Suzuki, T., *et al.*, J. *Med. Chem.* 42(15), 3001-3003 (1999); published PCT Application WO 01/18171 published on March 15, 2001 to Sloan-Kettering Institute for Cancer Research and The Trustees of Columbia University; published PCT Application WO 02/246144 to Hoffmann-La Roche; published PCT Application WO 02/22577 to Novartis; published PCT Application WO 02/30879 to Prolifix; published PCT Applications WO 01/38322 (published May 31, 2001), WO 01/70675 (published on September 27, 2001) and WO 00/71703 (published on November 30, 2000) all to Methylgene, Inc.; published PCT Application WO 00/21979 published on October 8, 1999 to Fujisawa Pharmaceutical Co., Ltd.; published PCT Application WO 98/40080 published on March 11, 1998 to Beacon Laboratories, L.L.C.; and Curtin M. (Current patent status of HDAC inhibitors *Expert Opin. Ther. Patents* (2002) 12(9): 1375-1384 and references cited therein).

SAHA or any of the other HDACs can be synthesized according to the methods outlined in the Experimental Details Section, or according to the method set forth in U.S. Patent Nos. 5,369,108, 5,700,811, 5,932,616 and 6,511,990, the contents of which are incorporated by reference in their entirety, or according to any other method known to a person skilled in the art.

Specific non-limiting examples of HDAC inhibitors are provided in the Table below. It should be noted that the present invention encompasses any compounds which are structurally similar to the compounds represented below, and which are capable of inhibiting histone deacetylases.

Name	**Structure**
MS-275	

Name	Structure
DEPSIPEPTIDE	
CI-994	
Apicidin	
A-161906	
Scriptaid	
PXD-101	
CHAP	
LAQ-824	

Name	Structure
Butyric Acid	O, HO
Depudecin	OH, O, O, OH
Oxamflatin	O, NHOH, $NHSO_2Ph$
Trichostatin C	O, O, NHOH, N

Stereochemistry

Many organic compounds exist in optically active forms having the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and 1 or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these compounds, called stereoisomers, are identical except that they are non-superimposable mirror images of one another. A specific stereoisomer can also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture.

Many of the compounds described herein can have one or more chiral centers and therefore can exist in different enantiomeric forms. If desired, a chiral carbon can be designated with an asterisk (*). When bonds to the chiral carbon are depicted as straight lines in the

formulas of the invention, it is understood that both the (R) and (S) configurations of the chiral carbon, and hence both enantiomers and mixtures thereof, are embraced within the formula. As is used in the art, when it is desired to specify the absolute configuration about a chiral carbon, one of the bonds to the chiral carbon can be depicted as a wedge (bonds to atoms above the
5 plane) and the other can be depicted as a series or wedge of short parallel lines is (bonds to atoms below the plane). The Cahn-Inglod-Prelog system can be used to assign the (R) or (S) configuration to a chiral carbon.

When the HDAC inhibitors of the present invention contain one chiral center, the compounds exist in two enantiomeric forms and the present invention includes both enantiomers
10 and mixtures of enantiomers, such as the specific 50:50 mixture referred to as a racemic mixtures. The enantiomers can be resolved by methods known to those skilled in the art, for example by formation of diastereoisomeric salts which may be separated, for example, by crystallization (see, CRC Handbook of Optical Resolutions via Diastereomeric Salt Formation by David Kozma (CRC Press, 2001)); formation of diastereoisomeric derivatives or complexes
15 which may be separated, for example, by crystallization, gas-liquid or liquid chromatography; selective reaction of one enantiomer with an enantiomer-specific reagent, for example enzymatic esterification; or gas-liquid or liquid chromatography in a chiral environment, for example on a chiral support for example silica with a bound chiral ligand or in the presence of a chiral solvent. It will be appreciated that where the desired enantiomer is converted into another chemical entity
20 by one of the separation procedures described above, a further step is required to liberate the desired enantiomeric form. Alternatively, specific enantiomers may be synthesized by asymmetric synthesis using optically active reagents, substrates, catalysts or solvents, or by converting one enantiomer into the other by asymmetric transformation.

Designation of a specific absolute configuration at a chiral carbon of the compounds of
25 the invention is understood to mean that the designated enantiomeric form of the compounds is in enantiomeric excess (ee) or in other words is substantially free from the other enantiomer. For example, the "R" forms of the compounds are substantially free from the "S" forms of the compounds and are, thus, in enantiomeric excess of the "S" forms. Conversely, "S" forms of the compounds are substantially free of "R" forms of the compounds and are, thus, in enantiomeric
30 excess of the "R" forms. Enantiomeric excess, as used herein, is the presence of a particular enantiomer at greater than 50%. For example, the enantiomeric excess can be about 60% or

more, such as about 70% or more, for example about 80% or more, such as about 90% or more. In a particular embodiment when a specific absolute configuration is designated, the enantiomeric excess of depicted compounds is at least about 90%. In a more particular embodiment, the enantiomeric excess of the compounds is at least about 95%, such as at least about 97.5%, for example, at least 99% enantiomeric excess.

When a compound of the present invention has two or more chiral carbons it can have more than two optical isomers and can exist in diastereoisomeric forms. For example, when there are two chiral carbons, the compound can have up to 4 optical isomers and 2 pairs of enantiomers ((S,S)/(R,R) and (R,S)/(S,R)). The pairs of enantiomers (e.g., (S,S)/(R,R)) are mirror image stereoisomers of one another. The stereoisomers which are not mirror-images (e.g., (S,S) and (R,S)) are diastereomers. The diastereoisomeric pairs may be separated by methods known to those skilled in the art, for example chromatography or crystallization and the individual enantiomers within each pair may be separated as described above. The present invention includes each diastereoisomer of such compounds and mixtures thereof.

As used herein, "a," an" and "the" include singular and plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an active agent" or "a pharmacologically active agent" includes a single active agent as well a two or more different active agents in combination, reference to "a carrier" includes mixtures of two or more carriers as well as a single carrier, and the like.

This invention is also intended to encompass pro-drugs of the HDAC inhibitors disclosed herein. A prodrug of any of the compounds can be made using well known pharmacological techniques.

This invention, in addition to the above listed compounds, is intended to encompass the use of homologs and analogs of such compounds. In this context, homologs are molecules having substantial structural similarities to the above-described compounds and analogs are molecules having substantial biological similarities regardless of structural similarities.

Tyrosine Kinase Inhibitors and Other Therapies

Recent developments have introduced, in addition to the traditional cytotoxic and hormonal therapies used to treat cancer, additional therapies for the treatment of cancer. For example, many forms of gene therapy are undergoing preclinical or clinical trials. In addition,

approaches are currently under development that are based on the inhibition of tumor vascularization (angiogenesis). The aim of this concept is to cut off the tumor from nutrition and oxygen supply provided by a newly built tumor vascular system. In addition, cancer therapy is also being attempted by the induction of terminal differentiation of the neoplastic cells. Suitable
5 differentiation agents include the compounds disclosed in any one or more of the following references, the contents of which are incorporated by reference herein.

A) Polar compounds (Marks *et al.* (1987); , Friend, C., Scher, W., Holland, J. W., and Sato, T. (1971) *Proc. Natl. Acad. Sci. (USA)* 68: 378-382; Tanaka, M., Levy, J., Terada, M., Breslow, R., Rifkind, R. A., and Marks, P. A. (1975) *Proc. Natl. Acad. Sci. (USA)* 72: 1003-
10 1006; Reuben, R. C., Wife, R. L., Breslow, R., Rifkind, R. A., and Marks, P. A. (1976) *Proc. Natl. Acad. Sci. (USA)* 73: 862-866); B) Derivatives of vitamin D and retinoic acid (Abe, E., Miyaura, C., Sakagami, H., Takeda, M., Konno, K., Yamazaki, T., Yoshika, S., and Suda, T. (1981) *Proc. Natl. Acad. Sci. (USA)* 78: 4990-4994; Schwartz, E. L., Snoddy, J. R., Kreutter, D., Rasmussen, H., and Sartorelli, A. C. (1983) *Proc. Am. Assoc. Cancer Res.* 24: 18; Tanenaga, K.,
15 Hozumi, M., and Sakagami, Y. (1980) *Cancer Res.* 40: 914-919); C) Steroid hormones (Lotem, J. and Sachs, L. (1975) *Int. J. Cancer 15:* 731-740); D) Growth factors (Sachs, L. (1978) *Nature (Lond.)* 274: 535, Metcalf, D. (1985) *Science*, 229: 16-22); E) Proteases (Scher, W., Scher, B. M., and Waxman, S. (1983) *Exp. Hematol.* 11: 490-498; Scher, W., Scher, B. M., and Waxman, S. (1982) *Biochem. & Biophys. Res. Comm.* 109: 348-354); F) Tumor promoters (Huberman, E.
20 and Callaham, M. F. (1979) *Proc. Natl. Acad. Sci. (USA)* 76: 1293-1297; Lottem, J. and Sachs, L. (1979) *Proc. Natl. Acad. Sci. (USA)* 76: 5158-5162); and G) Inhibitors of DNA or RNA synthesis (Schwartz, E. L. and Sartorelli, A. C. (1982) *Cancer Res. 42:* 2651-2655, Terada, M., Epner, E., Nudel, U., Salmon, J., Fibach, E., Rifkind, R. A., and Marks, P. A. (1978) *Proc. Natl. Acad. Sci. (USA)* 75: 2795-2799; Morin, M. J. and Sartorelli, A. C. (1984) *Cancer Res. 44*: 2807-
25 2812; Schwartz, E. L., Brown, B. J., Nierenberg, M., Marsh, J. C., and Sartorelli, A. C. (1983) *Cancer Res.* 43: 2725-2730; Sugano, H., Furusawa, M., Kawaguchi, T., and Ikawa, Y. (1973) *Bibl. Hematol.* 39: 943-954; Ebert, P. S., Wars, I., and Buell, D. N. (1976) *Cancer Res.* 36: 1809-1813; Hayashi, M., Okabe, J., and Hozumi, M. (1979) *Gann* 70: 235-238),

Tyrosine kinase inhibitors for use with the invention include all natural, recombinant, and
30 synthetic agents that decrease the activity or levels of one or more tyrosine kinases (for example, receptor tyrosine kinases), e.g., EGFR (ErbB-1; HER-1), HER-2/neu (ErbB-2), HER-3 (ErbB-3),

HER-4 (ErbB-4), discoidin domain receptor (DDR), ephrin receptor (EPHR), fibroblast growth factor receptor (FGFR), hepatocyte growth factor receptor (HGFR), insulin receptor (INSR), leukocytetyrosine kinase (Ltk/Alk), muscle-specific kinase (Musk), transforming growth factor receptor (e.g., TGFβ-RI and TGFβ-RII), platelet-derived growth factor receptor (PDGFR), and
5 vascular endothelial growth factor receptor (VEGFR). Inhibitors include endogenous or modified ligands for receptor tyrosine kinases such as epidermal growth factors (e.g., EGF), nerve growth factors (e.g., NGFα, NGFβ, NGFγ), heregulins (e.g., HRGα, HRGβ), transforming growth factors (e.g., TGFα, TGFβ), epiregulins (e.g., EP), amphiregulins (e.g., AR), betacellulins (e.g., BTC), heparin-binding EGF-like growth factors (e.g., HB-EGF), neuregulins (e.g., NRG-1,
10 NRG-2, NRG-4, NRG-4, also called glial growth factors), acetycholine receptor-inducing activity (ARIA), and sensory motor neuron-derived growth factors (SMDGF).

Other inhibitors include DMPQ (5,7-dimethoxy-3-(4-pyridinyl)quinoline dihydrochloride), Aminogenistein (4'-amino-6-hydroxyflavone), Erbstatin analog (2,5-dihydroxymethylcinnamate, methyl 2,5-dihydroxycinnamate), Imatinib (Gleevec ™, Glivec ™;
15 STI-571; 4-[(4-methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-yrimidinyl] amino]-phenyl]benzamide methanesulfonate), LFM-A13 (2-Cyano-N-(2,5-dibromophenyl)-3-hydroxy-2-butenamide), PD153035 (ZM 252868; 4-[(3-bromophenyl)amino]-6,7-dimethoxyquinazoline hydrochloride), Piceatannol (trans-3,3′,4,5′-tetrahydroxystilbene, 4-[(1E)-2-(3,5-dihydroxyphenyl)ethenyl]-1,2-benzenediol), PP1 (4-amino-5-(4-methylphenyl)-7-(t-
20 butyl)pyrazolo[3,4-d]pyrimidine), PP2 (4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo [3,4,d]pyrimidine), Pertuzumab (Omnitarg™; rhuMAb2C4), SU4312 (3-[[4-(dimethylamino) phenyl]methylene]-1,3-dihydro-2H-indol-2-one), SU6656 (2,3-dihydro-N,N-dimethyl-2-oxo-3-[(4,5,6,7-tetrahydro-1H-indol-2-yl)methylene]-1H-indole-5-sulfonamide), Bevacizumab (Avastin®; rhuMAb VEGF), Semaxanib (SU5416), SU6668 (Sugen, Inc.), and ZD6126
25 (Angiogene Pharmaceuticals). Included are inhibitors of EGFR, e.g., Cetuximab (Erbitux; IMC-C225; MoAb C225) and Gefitinib (IRESSA™; ZD1839; ZD1839; 4-(3-chloro-4-fluoroanilino)-7-methoxy-6-(3-morpholino propoxy)quinazoline), ZD6474 (AZD6474), , and EMD-72000 (Matuzumab), Panitumab (ABX-EGF; MoAb ABX-EGF;), ICR-62 (MoAb ICR-62), CI-1033 (PD183805; N-[-4-[(3-Chloro-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]-6-
30 quinazolinyl]-2-propenamide), Lapatinib (GW572016), AEE788 (pyrrolo-pyrimidine; Novartis), EKB-569 (Wyeth-Ayerst), and EXEL 7647/EXEL 09999 (EXELIS). Also included are Erlotinib

and derivatives, e.g., Tarceva®; NSC 718781, CP-358774, OSI-774, R1415; N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine, as represented by the structure:

or pharmaceutically acceptable salts or hydrates thereof (e.g., methanesulfonate salt, monohydrochloride).

Agents useful for the treatment of lung cancer (e.g., NSCLC) include the above-referenced inhibitors, as well as Pemetrexed (Alimta®), Bortezomib (Velcade®), Tipifarnib, Lonafarnib, BMS214662, Prinomastat, BMS275291, Neovastat, ISIS3521 (Affinitak™; LY900003), ISIS 5132, Oblimersen (Genasense®; G3139), and Carboxyamidotriazole (CAI) (see, e.g., Isobe T, *et al.*, *Semin. Oncol.* 32:315-328, 2005).

Alkylating Agents

Examples of alkylating agents include, but are not limited to, bischloroethylamines (nitrogen mustards, e.g., Chlorambucil, Cyclophosphamide, Ifosfamide, Mechlorethamine, Melphalan, uracil mustard), aziridines (e.g., Thiotepa), alkyl alkone sulfonates (e.g., Busulfan), nitrosoureas (e.g., Carmustine, Lomustine, Streptozocin), nonclassic alkylating agents (Altretamine, Dacarbazine, and Procarbazine), platinum compounds (Carboplastin and Cisplatin). These compounds react with phosphate, amino, hydroxyl, sulfihydryl, carboxyl, and imidazole groups.

Under physiological conditions, these drugs ionize and produce positively charged ion that attach to susceptible nucleic acids and proteins, leading to cell cycle arrest and/or cell death. The alkylating agents are cell cycle phase nonspecific agents because they exert their activity independently of the specific phase of the cell cycle. The nitrogen mustards and alkyl alkone sulfonates are most effective against cells in the Gl or M phase. Nitrosoureas, nitrogen mustards, and aziridines impair progression from the G1 and S phases to the M phases. Chabner and

Collins eds. (1990) "Cancer Chemotherapy: Principles and Practice", Philadelphia: JB Lippincott.

The alkylating agents are active against wide variety of neoplastic diseases, with significant activity in the treatment of leukemias and lymphomas as well as solid tumors. Clinically this group of drugs is routinely used in the treatment of acute and chronic leukemias; Hodgkin's disease; non-Hodgkin's lymphoma; multiple myeloma; primary brain tumors; carcinomas of the breast, ovaries, testes, lungs, bladder, cervix, head and neck, and malignant melanoma.

Antibiotic Agents

Antibiotics (e.g., cytotoxic antibiotics) act by directly inhibiting DNA or RNA synthesis and are effective throughout the cell cycle. Examples of antibiotic agents include anthracyclines (e.g., Doxorubicin, Daunorubicin, Epirubicin, Idarubicin, and Anthracenedione), Mitomycin C, Bleomycin, Dactinomycin, Plicatomycin. These antibiotic agents interfere with cell growth by targeting different cellular components. For example, anthracyclines are generally believed to interfere with the action of DNA topoisomerase II in the regions of transcriptionally active DNA, which leads to DNA strand scissions.

Bleomycin is generally believed to chelate iron and forms an activated complex, which then binds to bases of DNA, causing strand scissions and cell death.

The antibiotic agents have been used as therapeutics across a range of neoplastic diseases, including carcinomas of the breast, lung, stomach and thyroids, lymphomas, myelogenous leukemias, myelomas, and sarcomas.

Antimetabolic Agents

Antimetabolic agents (i.e., antimetabolites) are a group of drugs that interfere with metabolic processes vital to the physiology and proliferation of cancer cells. Actively proliferating cancer cells require continuous synthesis of large quantities of nucleic acids, proteins, lipids, and other vital cellular constituents.

Many of the antimetabolites inhibit the synthesis of purine or pyrimidine nucleosides or inhibit the enzymes of DNA replication. Some antimetabolites also interfere with the synthesis of ribonucleosides and RNA and/or amino acid metabolism and protein synthesis as well. By

interfering with the synthesis of vital cellular constituents, antimetabolites can delay or arrest the growth of cancer cells. Antimitotic agents are included in this group. Examples of antimetabolic agents include, but are not limited to, Fluorouracil (5-FU), Floxuridine (5-FUdR), Methotrexate, Leucovorin, Hydroxyurea, Thioguanine (6-TG), Mercaptopurine (6-MP), Cytarabine, Pentostatin, Fludarabine Phosphate, Cladribine (2-CDA), Asparaginase, and Gemcitabine.

Antimetabolic agents have been widely used to treat several common forms of cancer including carcinomas of colon, rectum, breast, liver, stomach and pancreas, malignant melanoma, acute and chronic leukemia and hair cell leukemia.

Hormonal Agents

The hormonal agents are a group of drug that regulate the growth and development of their target organs. Most of the hormonal agents are sex steroids and their derivatives and analogs thereof, such as estrogens, progestogens, anti-estrogens, androgens, anti-androgens and progestins. These hormonal agents may serve as antagonists of receptors for the sex steroids to down regulate receptor expression and transcription of vital genes. Examples of such hormonal agents are synthetic estrogens (e.g., Diethylstibestrol), antiestrogens (e.g., Tamoxifen, Toremifene, Fluoxymesterol, and Raloxifene), antiandrogens (e.g., Bicalutamide, Nilutamide, and Flutamide), aromatase inhibitors (e.g., Aminoglutethimide, Anastrozole, and Tetrazole), luteinizing hormone release hormone (LHRH) analogues, Ketoconazole, Goserelin Acetate, Leuprolide, Megestrol Acetate, and Mifepristone.

Hormonal agents are used to treat breast cancer, prostate cancer, melanoma, and meningioma. Because the major action of hormones is mediated through steroid receptors, 60% receptor-positive breast cancer responded to first-line hormonal therapy; and less than 10% of receptor-negative tumors responded. The main side effect associated with hormonal agents is flare. The frequent manifestations are an abrupt increase of bone pain, erythema around skin lesions, and induced hypercalcemia.

Specifically, progestogens are used to treat endometrial cancers, since these cancers occur in women that are exposed to high levels of oestrogen unopposed by progestogen.

Antiandrogens are used primarily for the treatment of prostate cancer, which is hormone dependent. They are used to decrease levels of testosterone, and thereby inhibit growth of the tumor.

Hormonal treatment of breast cancer involves reducing the level of oestrogen-dependent activation of oestrogen receptors in neoplastic breast cells. Anti-oestrogens act by binding to oestrogen receptors and prevent the recruitment of coactivators, thus inhibiting the oestrogen signal.

5 LHRH analogues are used in the treatment of prostate cancer to decrease levels of testosterone and so decrease the growth of the tumor.

Aromatase inhibitors act by inhibiting the enzyme required for hormone synthesis. In post-menopausal women, the main source of oestrogen is through the conversion of androstenedione by aromatase.

10

Plant-derived Agents

Plant-derived agents are a group of drugs that are derived from plants or modified based on the molecular structure of the agents. They inhibit cell replication by preventing the assembly of the cell's components that are essential to cell division.

15 Examples of plant derived agents include vinca alkaloids (e.g., Vincristine, Vinblastine, Vindesine, Vinzolidine, and Vinorelbine), podophyllotoxins (e.g., Etoposide (VP-16) and Teniposide (VM-26)), and taxanes (e.g., Paclitaxel and Docetaxel). These plant-derived agents generally act as antimitotic agents that bind to tubulin and inhibit mitosis. Podophyllotoxins such as etoposide are believed to interfere with DNA synthesis by interacting with topoisomerase

20 II, leading to DNA strand scission.

Plant-derived agents are used to treat many forms of cancer. For example, vincristine is used in the treatment of the leukemias, Hodgkin's and non-Hodgkin's lymphoma, and the childhood tumors neuroblastoma, rhabdomyosarcoma, and Wilms' tumor. Vinblastine is used against the lymphomas, testicular cancer, renal cell carcinoma, mycosis fungoides, and Kaposi's

25 sarcoma. Doxetaxel has shown promising activity against advanced breast cancer, non-small cell lung cancer (NSCLC), and ovarian cancer.

Etoposide is active against a wide range of neoplasms, of which small cell lung cancer, testicular cancer, and NSCLC are most responsive.

Biologic Agents

Biologic agents are a group of biomolecules that elicit cancer/tumor regression when used alone or in combination with chemotherapy and/or radiotherapy. Examples of biologic agents include immunomodulating proteins such as cytokines, monoclonal antibodies against tumor antigens, tumor suppressor genes, and cancer vaccines.

Cytokines possess profound immunomodulatory activity. Some cytokines such as interleukin-2 (IL-2, Aldesleukin) and interferon-α (IFN-α) demonstrated antitumor activity and have been approved for the treatment of patients with metastatic renal cell carcinoma and metastatic malignant melanoma. IL-2 is a T-cell growth factor that is central to T-cell-mediated immune responses. The selective antitumor effects of IL-2 on some patients are believed to be the result of a cell-mediated immune response that discriminate between self and nonself.

Interferon-α includes more than 23 related subtypes with overlapping activities. IFN-α has demonstrated activity against many solid and hematologic malignancies, the later appearing to be particularly sensitive.

Examples of interferons include interferon-α, interferon-β (fibroblast interferon) and interferon-γ (lymphocyte interferon). Examples of other cytokines include erythropoietin (Epoietin-α; EPO), granulocyte-CSF (Filgrastin), and granulocyte, macrophage-CSF (Sargramostim). Other immuno-modulating agents other than cytokines include bacillus Calmette-Guerin, levamisole, and octreotide, a long-acting octapeptide that mimics the effects of the naturally occurring hormone somatostatin.

Furthermore, the anti-cancer treatment can comprise treatment by immunotherapy with antibodies and reagents used in tumor vaccination approaches. The primary drugs in this therapy class are antibodies, alone or carrying e.g. toxins or chemostherapeutics/cytotoxics to cancer cells. Monoclonal antibodies against tumor antigens are antibodies elicited against antigens expressed by tumors, particularly tumor-specific antigens. For example, monoclonal antibody HERCEPTIN® (Trastuzumab) is raised against human epidermal growth factor receptor2 (HER2) that is overexpressed in some breast tumors including metastatic breast cancer. Overexpression of HER2 protein is associated with more aggressive disease and poorer prognosis in the clinic. HERCEPTIN® is used as a single agent for the treatment of patients with metastatic breast cancer whose tumors over express the HER2 protein.

Another example of monoclonal antibodies against tumor antigens is RITUXAN® (Rituximab) that is raised against CD20 on lymphoma cells and selectively deplete normal and malignant CD20+ pre-B and mature B cells.

RITUXAN is used as single agent for the treatment of patients with relapsed or refractory low-grade or follicular, CD20+, B cell non-Hodgkin's lymphoma. MYELOTARG® (Gemtuzumab Ozogamicin) and CAMPATH® (Alemtuzumab) are further examples of monoclonal antibodies against tumor antigens that may be used.

Endostatin is a cleavage product of plasminogen used to target angiogenesis.

Tumor suppressor genes are genes that function to inhibit the cell growth and division cycles, thus preventing the development of neoplasia. Mutations in tumor suppressor genes cause the cell to ignore one or more of the components of the network of inhibitory signals, overcoming the cell cycle checkpoints and resulting in a higher rate of controlled cell growth-cancer. Examples of the tumor suppressor genes include Duc-4, NF-1, NF-2, RB, p53, WT1, BRCA1, and BRCA2.

DPC4 is involved in pancreatic cancer and participates in a cytoplasmic pathway that inhibits cell division. NF-1 codes for a protein that inhibits Ras, a cytoplasmic inhibitory protein. NF-1 is involved in neurofibroma and pheochromocytomas of the nervous system and myeloid leukemia. NF-2 encodes a nuclear protein that is involved in meningioma, schwanoma, and ependymoma of the nervous system. RB codes for the pRB protein, a nuclear protein that is a major inhibitor of cell cycle. RB is involved in retinoblastoma as well as bone, bladder, small cell lung and breast cancer. P53 codes for p53 protein that regulates cell division and can induce apoptosis. Mutation and/or inaction of p53 is found in a wide range of cancers. WTI is involved in Wilms' tumor of the kidneys. BRCA1 is involved in breast and ovarian cancer, and BRCA2 is involved in breast cancer. The tumor suppressor gene can be transferred into the tumor cells where it exerts its tumor suppressing functions.

Cancer vaccines are a group of agents that induce the body's specific immune response to tumors. Most of cancer vaccines under research and development and clinical trials are tumor-associated antigens (TAAs). TAAs are structures (i.e., proteins, enzymes, or carbohydrates) that are present on tumor cells and relatively absent or diminished on normal cells. By virtue of being fairly unique to the tumor cell, TAAs provide targets for the immune system to recognize and cause their destruction. Examples of TAAs include gangliosides (GM2), prostate specific

antigen (PSA), α-fetoprotein (AFP), carcinoembryonic antigen (CEA) (produced by colon cancers and other adenocarcinomas, e.g., breast, lung, gastric, and pancreatic cancers), melanoma-associated antigens (MART-1, gap100, MAGE 1,3 tyrosinase), papillomavirus E6 and E7 fragments, whole cells or portions/lysates of autologous tumor cells and allogeneic tumor
5 cells.

Retinoids or retinoid agents for use with the invention include all natural, recombinant, and synthetic derivatives or mimetics of vitamin A, for example, retinyl palmitate, retinoyl-beta-glucuronide (vitamin A1 beta-glucuronide), retinyl phosphate (vitamin A1 phosphate), retinyl esters, 4-oxoretinol, 4-oxoretinaldehyde, 3-dehydroretinol (vitamin A2), 11-cis-retinal (11-cis-
10 retinaldehyde, 11-cis or neo b vitamin A1 aldehyde), 5,6-epoxyretinol (5,6-epoxy vitamin A1 alcohol), anhydroretinol (anhydro vitamin A1) and 4-ketoretinol (4-keto-vitamin A1 alcohol), all-trans retinoic acid (ATRA; Tretinoin; vitamin A acid; 3,7-dimethyl-9-(2,6,6,-trimethyl-1-cyclohenen-1-yl)-2,4,6,8-nonatetraenoic acid [CAS No. 302-79-4]), lipid formulations of all-trans retinoic acid (e.g., ATRA-IV), 9-cis retinoic acid (9-cis-RA; Alitretinoin; Panretin©;
15 LGD1057), (e)-4-[2-(5,6,7,8-tetrahydro-2-naphthalenyl)-1-propenyl]-benzoic acid, 3-methyl-(E)-4-[2-(5,6,7,8-tetrahydro-2-naphthalenyl)-1-propenyl]-benzoic acid, Fenretinide (N-(4-hydroxyphenyl)retinamide; 4-HPR), Etretinate (2,4,6,8-nonatetraenoic acid), Acitretin (Ro 10-1670), Tazarotene (ethyl 6-[2-(4,4-dimethylthiochroman-6-yl)-ethynyl] nicotinate), Tocoretinate (9-cis-tretinoin tocoferil), Adapalene (6-[3-(1-adamantyl)-4-methoxyphenyl]-2-naphthoic acid),
20 Motretinide (trimethylmethoxyphenyl-N-ethyl retinamide), and retinaldehyde.

Also included as retinoids are retinoid related molecules such as CD437 (also called 6-[3-(1-adamantyl)-4-hydroxphenyl]-2-naphthalene carboxylic acid and AHPN), CD2325, ST1926 ([E-3-(4'-hydroxy-3'-adamantylbiphenyl-4-yl)acrylic acid), ST1878 (methyl 2-[3-[2-[3-(2-methoxy-1,1-dimethyl-2-oxoethoxy)pheno-xy]ethoxy]phenoxy]isobutyrate), ST2307, ST1898,
25 ST2306, ST2474, MM11453, MM002 (3-Cl-AHPC), MX2870-1, MX3350-1, MX84, and MX90-1 (Garattini *et al.*, 2004, *Curr. Pharmaceut. Design* 10:433-448; Garattini and Terao, 2004, *J. Chemother.* 16:70-73). Included for use with the invention are retinoid agents that bind to one or more RXR. Also included are retinoid agents that bind to one or more RXR and do not bind to one or more RAR (i.e., selective binding to RXR; rexinoids), e.g., docosahexanoic acid
30 (DHA), phytanic acid, methoprene acid, LG100268 (LG268), LG100324, LGD1057, SR11203, SR11217, SR11234, SR11236, SR11246, AGN194204 (see, e.g., Simeone and Tari, 2004, *Cell*

Mol. Life Sci. 61:1475-1484; Rigas and Dragnev, 2005, *The Oncologist* 10:22-33; Ahuja *et al.*, 2001, *Mol. Pharmacol.* 59:765-773; Gorgun and Foss, 2002, *Blood* 100:1399-1403; Bischoff *et al.*, 1999, *J. Natl. Cancer Inst.* 91:2118- 2123; Sun *et al.*, 1999, *Clin. Cancer Res.* 5:431-437; Crow and Chandraratna, 2004, *Breast Cancer Res.* 6:R546-R555). Further included are
5 derivatives of 9-cis-RA. Particularly included are 3-methyl TTNEB and related agents, e.g., Targretin®; Bexarotene; LGD1069; 4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl) ethenyl] benzoic acid, or a pharmaceutically acceptable salt or hydrate thereof.

Other Agents

10 Other agents may also be useful for use with the present invention, for example, for adjunct therapies. Such adjunctive agents can be used to enhance the effectiveness of anticancer agents or to prevent or treat conditions associated with anticancer agents such as low blood counts, hypersensitivity reactions, neutropenia, anemia, thrombocytopenia, hypercalcemia, mucositis, bruising, bleeding, toxicity (e.g., Leucovorin), fatigue, pain, nausea, and vomiting.
15 Antiemetic agents (e.g., 5-HT receptor blockers or benzodiazepines), anti-inflammatory agents (e.g., adrenocortical steroids or antihistamines), dietary supplements (e.g., folic acid), vitamins (e.g., Vitamin E, Vitamin C, Vitamin B_6, Vitamin B_{12}), and acid reducing agents (e.g., H_2 receptor blockers) can be useful for increasing patient tolerance to cancer therapy. Examples of H_2 receptor blockers include Ranitidine, Famotidine, and Cimetidine. Examples of
20 antihistamines include Diphenhydramine, Clemastine, Chlorpheniramine, Chlorphenamine, Dimethindene maleate, and Promethazine. Examples of steroids include Dexamethasone, Hydrocortisone, and Prednisone. Other agents include growth factors such as epoetin alpha (e.g., Procrit®, Epogen®) for stimulating red blood cell production, G-CSF (granulocyte colony-stimulating factor; filgrastim, e.g., Neupogen®) for stimulating neutrophil production, GM-CSF
25 (granulocyte-macrophage colony-stimulating factor) for stimulating production of several white blood cells, including macrophages, and IL-11 (interleukin-11, e.g., Neumega®) for stimulating production of platelets.

Leucovorin (e.g., Leucovorin calcium, Roxane Laboratories, Inc., Columbus, OH; also called folinic acid, calcium folinate, citrovorum factor) can be used as an antidote to folic acid
30 antagonists, and can also potentiate the activity of certain drugs, such as Fluorouracil.

Leucovorin calcium is the calcium salt of N-[4-[[(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid.

Dexamethasone (e.g., Decadron®; Merck & Co., Inc., Whitehouse Station, NJ) is a synthetic adrenocortical steroid that can be used as an anti-inflammatory agent to control allergic reactions, e.g., drug hypersensitivity reactions. Dexamethasone tablets for oral administration comprise 9-fluoro-11-beta,17,21-trihydroxy-16-alpha-methylpregna-1,4-diene-3,20-dione, as represented by the structure:

.

Dexamethasone phosphate for intravenous administration comprises 9-fluoro-11β,17-dihydroxy-16α-methyl-21-(phosphonooxy)pregna-1,4-diene-3,20-dione disodium salt, as represented by the structure:

.

Diphenhydramine (e.g., Benadryl®; Parkedale Pharmaceuticals, Inc., Rochester, MI) is an antihistamine drug used for amelioration of allergic reactions. Diphenhydramine hydrochloride (e.g., Diphenhydramine HCl for injection) is 2-(diphenylmethoxy)-N,N-dimethylethylamine hydrochloride, as represented by the structure:

Ranitidine (e.g., Zantac®; GlaxoSmithKline, Research Triangle Park, NC) is a competitive inhibitor of histamine at histamine H_2-receptors, and can be used to reduce stomach acid. Ranitidine hydrochloride (e.g., tablets or injection) is N[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]-N′-methyl-2-nitro-1,1-ethenediamine, HCl, as represented by the structure:

Cimetidine (e.g., Tagamet®; GlaxoSmithKline, Research Triangle Park, NC) is also a competitive inhibitor of histamine at histamine H2 receptors, and can be used to reduce stomach acid. Cimetidine is *N″*-cyano-*N*-methyl-*N′*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]-ethyl]-guanidine, as represented by the structure:

Aprepitant (e.g., EMEND®; Merck & Co., Inc.) is a substance P/neurokinin 1 (NK1) receptor antagonist and antiemetic. Aprepitant is 5-[[(2*R*,3*S*)-2-[(1*R*)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]methyl]-1,2-dihydro-3*H*-1,2,4-triazol-3-one, as represented by the structure:

•

Ondansetron (e.g., Zofran®; GlaxoSmithKline, Research Triangle Park, NC) is a selective blocking agent of 5-HT3 serotonin receptor and antiemetic. Ondansetron hydrochloride (e.g., for injection) is (±)1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl)methyl]-
5 4H-carbazol-4-one, monohydrochloride, dihydrate, as represented by the structure:

•HCl•$2H_2O$

•

Lorazepam (e.g., Lorazepam Injection; Baxter Healthcare Corp., Deerfield, IL), is a benzodiazepine with anticonvulsant effects. Lorazepam is 7-chloro-5(2-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one, as represented by the structure:

10 •

The use of all of these approaches in combination with HDAC inhibitors, e.g. SAHA, is within the scope of the present invention.

Administration of the HDAC Inhibitor

Routes of Administration

The HDAC inhibitor (e.g. SAHA), can be administered by any known administration method known to a person skilled in the art. Examples of routes of administration include but are not limited to oral, parenteral, intraperitoneal, intravenous, intraarterial, transdermal, topical, sublingual, intramuscular, rectal, transbuccal, intranasal, liposomal, via inhalation, vaginal, intraoccular, via local delivery by catheter or stent, subcutaneous, intraadiposal, intraarticular, intrathecal, or in a slow release dosage form. SAHA or any one of the HDAC inhibitors can be administered in accordance with any dose and dosing schedule that, together with the effect of one or more anti-cancer agents, achieves a dose effective to treat disease.

Of course, the route of administration of SAHA or any one of the other HDAC inhibitors is independent of the route of administration of one or more anti-cancer agents. A particular route of administration for SAHA is oral administration. Thus, in accordance with this embodiment, SAHA is administered orally, the second anti-cancer agent, Erlotinib, and optional third anti-cancer agent can be administered orally, parenterally, intraperitoneally, intravenously, intraarterially, transdermally, sublingually, intramuscularly, rectally, transbuccally, intranasally, liposomally, via inhalation, vaginally, intraoccularly, via local delivery by catheter or stent, subcutaneously, intraadiposally, intraarticularly, intrathecally, or in a slow release dosage form.

As examples, the HDAC inhibitors of the invention can be administered in such oral forms as tablets, capsules (each of which includes sustained release or timed release formulations), pills, powders, granules, elixirs, tinctures, suspensions, syrups, and emulsions. Likewise, the HDAC inhibitors can be administered by intravenous (e.g., bolus or infusion), intraperitoneal, subcutaneous, intramuscular, or other routes using forms well known to those of ordinary skill in the pharmaceutical arts. A particular route of administration of the HDAC inhibitor is oral administration.

The HDAC inhibitors can also be administered in the form of a depot injection or implant preparation, which may be formulated in such a manner as to permit a sustained release of the active ingredient. The active ingredient can be compressed into pellets or small cylinders and implanted subcutaneously or intramuscularly as depot injections or implants. Implants may employ inert materials such as biodegradable polymers or synthetic silicones, for example, Silastic, silicone rubber or other polymers manufactured by the Dow-Corning Corporation.

The HDAC inhibitor can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines. Liposomal preparations of tyrosine kinase inhibitors may also be used in
5 the methods of the invention. Liposome versions of tyrosine kinase inhibitors may be used to increase tolerance to the inhibitors.

The HDAC inhibitors can also be delivered by the use of monoclonal antibodies as individual carriers to which the compound molecules are coupled.

The HDAC inhibitors can also be prepared with soluble polymers as targetable drug
10 carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxy-propyl-methacrylamide-phenol, polyhydroxyethyl-aspartamide-phenol, or polyethyleneoxide-polylysine substituted with palmitoyl residues. Furthermore, the HDAC inhibitors can be prepared with biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyglycolic acid, copolymers of polylactic and polyglycolic acid,
15 polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and cross linked or amphipathic block copolymers of hydrogels.

In a specific embodiment, the HDAC inhibitor, e.g. SAHA, is administered orally in a gelatin capsule, which can comprise excipients such as microcrystalline cellulose, croscarmellose
20 sodium and magnesium stearate. A further embodiment includes 200 mg of solid SAHA with 89.5 mg of microcrystalline cellulose, 9 mg of sodium croscarmellose, and 1.5 mg of magnesium stearate contained in a gelatin capsule.

Dosages and Dosage Schedules

25 The dosage regimen utilizing the HDAC inhibitors can be selected in accordance with a variety of factors including type, species, age, weight, sex and the type of disease being treated; the severity (i.e., stage) of the disease to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound or salt thereof employed. A dosage regimen can be used, for example, to prevent, inhibit (fully or partially), or arrest the progress of
30 the disease.

In accordance with the invention, an HDAC inhibitor (e.g., SAHA or a pharmaceutically acceptable salt or hydrate thereof) can be administered by continuous or intermittent dosages. For example, intermittent administration of an HDAC inhibitor may be administration one to six days per week or it may mean administration in cycles (e.g. daily administration for two to eight consecutive weeks, then a rest period with no administration for up to one week) or it may mean administration on alternate days. The HDAC inhibitors may be administered in cycles, with rest periods in between the cycles (e.g. treatment for two to eight weeks with a rest period of up to a week between treatments). In some embodiments of the present invention, the HDAC inhibitor can be administered according to the dosages and dosing schedules described herein as a pharmaceutical composition, either together or separately with the tyrosine kinase inhibitor (and optionally, with another anti-cancer agent).

For example, SAHA or any one of the HDAC inhibitors can be administered in a total daily dose of up to 800 mg. The HDAC inhibitor can be administered once daily (QD), or divided into multiple daily doses such as twice daily (BID), and three times daily (TID). The HDAC inhibitor can be administered at a total daily dosage of up to 800 mg, e.g., 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 700 mg, or 800 mg, which can be administered in one daily dose or can be divided into multiple daily doses as described above. In specific aspects, the administration is oral.

In one embodiment, the HDAC inhibitor is administered once daily at a dose of about 200-600 mg. In another embodiment, the HDAC inhibitor is administered twice daily at a dose of about 200-400 mg. In another embodiment, the HDAC inhibitor is administered twice daily at a dose of about 200-400 mg intermittently, for example three, four or five days per week. In one embodiment, the daily dose is 200 mg which can be administered once-daily, twice-daily or three-times daily. In one embodiment, the daily dose is 300 mg which can be administered once-daily, twice-daily or three-times daily. In one embodiment, the daily dose is 400 mg which can be administered once-daily, twice-daily or three-times daily.

SAHA or any one of the HDAC inhibitors can be administered in accordance with any dose and dosing schedule that, together with the effect of the anti-cancer agent, achieves a dose effective to treat cancer. The HDAC inhibitors can be administered in a total daily dose that may vary from patient to patient, and may be administered at varying dosage schedules. For example, SAHA or any of the HDAC inhibitors can be administered to the patient at a total daily dosage of

between 25-4000 mg/m^2. In particular, SAHA or any one of the HDAC inhibitors can be administered in a total daily dose of up to 800 mg, especially by oral administration, once, twice or three times daily, continuously (every day) or intermittently (e.g., 3-5 days a week). In addition, the administration can be continuous, i.e., every day, or intermittently.

5 A particular treatment protocol comprises continuous administration (i.e., every day), once, twice or three times daily at a total daily dose in the range of about 200 mg to about 600 mg. Another treatment protocol comprises intermittent administration of between three to five days a week, once, twice or three times daily at a total daily dose in the range of about 200 mg to about 600 mg.

10 The HDAC inhibitor can be administered continuously once daily at a dose of 400 mg or twice daily at a dose of 200 mg. Alternatively, the HDAC inhibitor can be administered intermittently three days a week, once daily at a dose of 400 mg or twice daily at a dose of 200 mg. The HDAC inhibitor can be administered intermittently four days a week, once daily at a dose of 400 mg or twice daily at a dose of 200 mg. The HDAC inhibitor can also be
15 administered intermittently five days a week, once daily at a dose of 400 mg or twice daily at a dose of 200 mg.

For example, the HDAC inhibitor can be administered continuously once daily at a dose of 600 mg, twice daily at a dose of 300 mg, or three times daily at a dose of 200 mg. In one embodiment, the HDAC inhibitor, e.g., SAHA, is administered continuously at a once-daily dose
20 of 300 mg. Alternatively, the HDAC inhibitor can be administered intermittently three days a week, once daily at a dose of 600 mg, twice daily at a dose of 300 mg, or three times daily at a dose of 200 mg. The HDAC inhibitor can also be administered intermittently four days a week, once daily at a dose of 600 mg, twice daily at a dose of 300 mg, or three times daily at a dose of 200 mg. The HDAC inhibitor can also be administered intermittently five days a week, once
25 daily at a dose of 600 mg, twice daily at a dose of 300 mg, or three times daily at a dose of 200 mg.

In addition, the HDAC inhibitor may be administered according to any of the schedules described above, consecutively for a few weeks, followed by a rest period. For example, the HDAC inhibitor may be administered according to any one of the schedules described above
30 from two to eight weeks, followed by a rest period of one week. The HDAC inhibitor may also be administered three times daily for two consecutive weeks, followed by one week of rest.

The HDAC inhibitor can be administered continuously (i.e., daily) or intermittently (e.g., at least 3 days per week) with a once daily dose of about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, or about 800 mg. In one particular embodiment, the HDAC inhibitor is continuously administered once daily at a dose of 300 mg.

5 In other embodiments, the HDAC inhibitor is administered once daily at a dose of about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, or about 800 mg for at least one period of 3 out of 7 days (e.g., 3 consecutive days with dosage followed by 4 consecutive days without dosage). Preferably, the HDAC inhibitor is administered once daily at a dose of 200 mg for at least one period of 3 out of 7 days. In another embodiment, the HDAC 10 inhibitor is administered once daily at a dose of 300 mg for at least one period of 3 out of 7 days. In yet another embodiment, the HDAC inhibitor is administered once daily at a dose of 400 mg for at least one period of 3 out of 7 days. In other embodiments, the HDAC inhibitor is administered once daily at a dose of 500 mg for at least one period of 3 out of 7 days. In such dosing regimens, the administration can be repeated weekly, or administered for one week, 15 followed by a one week, two week, or three week rest period. Alternatively, the HDAC inhibitor can be administered for two weeks, followed by a two-week rest period, or can be administered for three weeks, followed by a one week rest period.

The HDAC inhibitor can be administered once daily at a dose of about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, or about 800 mg for at least 20 one period of 7 out of 21 days (e.g., 7 consecutive days or Days 1-7 in a 21 day cycle), or for at least one period of 14 out of 21 days (e.g., 14 consecutive days or Days 1-14 in a 21 day cycle), or for at least one period of 14 out of 28 days (e.g., 14 consecutive days or Days 1-14 of a 28 day cycle). In one particular embodiment, the HDAC inhibitor is administered once daily at a dose of 300 mg for at least one period of 14 out of 28 days.

25 In another embodiment, the HDAC inhibitor is administered once daily at a dose of about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, or about 800 mg for example, for at least one period of 21 out of 28 days (e.g., 21 consecutive days or Days 1-21 in a 28 day cycle).

The HDAC inhibitors of the present invention can be administered continuously (i.e., 30 daily) or intermittently (e.g., at least 3 days per week) with a twice daily dose of about 200 mg, about 250 mg, about 300 mg, or about 400 mg.

In one embodiment, the HDAC inhibitor is administered twice daily at a dose of about 200 mg, about 250 mg, about 300 mg, or about 400 mg (per dose) for at least one period of 3 out of 7 days (e.g., 3 consecutive days with dosage followed by 4 consecutive days without dosage). In a particular embodiment, the HDAC inhibitor is administered twice daily at a dose of 200 mg for at least one period of 3 out of 7 days. In another embodiment, the HDAC inhibitor is administered twice daily at a dose of 300 mg for at least one period of 3 out of 7 days.

Alternatively, the HDAC inhibitor can be administered twice daily at a dose of about 200 mg, about 250 mg, about 300 mg, or about 400 mg (per dose) for at least one period of 4 out of 7 days (e.g., 4 consecutive days with dosage followed by 3 consecutive days without dosage).

The HDAC inhibitor can also be administered twice daily at a dose of about 200 mg, about 250 mg, about 300 mg, or about 400 mg (per dose) for at least one period of 5 out of 7 days (e.g., 5 consecutive days with dosage followed by 2 consecutive days without dosage). In some embodiments, the HDAC inhibitor is administered twice daily at a dose of about 200 mg, about 250 mg, about 300 mg, or about 400 mg (per dose) for at least one period of 3 out of 7 days in a cycle of 21 days (e.g., 3 consecutive days or Days 1-3 for up to 3 weeks in a 21 day cycle). In one particular embodiment, the HDAC inhibitor is administered twice daily at a dose of about 200 mg, about 250 mg, about 300 mg, or about 400 mg (per dose), for example, for one period of 3 out of 7 days in a cycle of 21 days (e.g., 3 consecutive days or Days 1-3 in a 21 day cycle). Alternatively, the HDAC inhibitor can be administered twice daily at a dose of about 200 mg, about 250 mg, about 300 mg, or about 400 mg (per dose), for example, for at least two periods of 3 out of 7 days in a cycle of 21 days (e.g., 3 consecutive days or Days 1-3 and Days 8-10 for Week 1 and Week 2 of a 21 day cycle), or for example, for at least three periods of 3 out of 7 days in a cycle of 21 days (e.g., 3 consecutive days or Days 1-3, Days 8-10, and Days 15-17 for Week 1, Week 2, and Week 3 of a 21 day cycle). In other embodiments, the HDAC inhibitor is administered twice daily at a dose of about 200 mg, about 250 mg, about 300 mg, or about 400 mg (per dose), for at least one period of 4 out of 7 days in a cycle of 21 days (e.g., 4 consecutive days or Days 1-4 for up to 3 weeks in a 21 day cycle), or for at least one period of 5 out of 7 days in a cycle of 21 days (e.g., 5 consecutive days or Days 1-5 for up to 3 weeks in a 21 day cycle)

The HDAC inhibitor can also be administered twice daily at a dose of about 200 mg, about 250 mg, about 300 mg, or about 400 mg (per dose) for at least one period of 3 out of 7

days in a cycle of 28 days (e.g., 3 consecutive days or Days 1-3 for up to 4 weeks in a 28 day cycle).

In addition, the HDAC inhibitor can alternatively be administered twice daily at a dose of about 200 mg, about 250 mg, about 300 mg, or about 400 mg (per dose) for at least two, three, or four periods of 3 out of 7 days in a cycle of 28 days (e.g., 3 consecutive days or Days 1-3, Days 8-10, Days 15-17, and Days 22-24 for Week 1, Week 2, Week 3, and Week 4 in a 28 day cycle).

In one embodiment, the HDAC inhibitor is administered twice daily at a dose of about 200 mg, about 250 mg, about 300 mg, or about 400 mg (per dose), for example, for at least one period of 7 out of 14 days (e.g., 7 consecutive days or Days 1-7 in a 14 day cycle). In a particular embodiment, the HDAC inhibitor is administered twice daily at a dose of 300 mg for at least one period of 7 out of 14 days.

The HDAC inhibitor can be administered twice daily at a dose of bout 200 mg, about 250 mg, about 300 mg, or about 400 mg (per dose), for example, for at least one period of 14 out of 21 days (e.g., 14 consecutive days or Days 1-14 in a 21 day cycle). The HDAC inhibitor can also be administered twice daily at a dose of about 200 mg, about 250 mg, about 300 mg, or about 400 mg (per dose) for at least one period of 14 out of 28 days (e.g., 14 consecutive days of Days 1-14 in a 28 day cycle).

Intravenously or subcutaneously, the patient would receive the HDAC inhibitor in quantities sufficient to deliver between about 3-1500 mg/m^2 per day, for example, about 3, 30, 60, 90, 180, 300, 600, 900, 1200 or 1500 mg/m^2 per day. Such quantities may be administered in a number of suitable ways, e.g. large volumes of low concentrations of HDAC inhibitor during one extended period of time or several times a day. The quantities can be administered for one or more consecutive days, intermittent days or a combination thereof per week (7 day period). Alternatively, low volumes of high concentrations of HDAC inhibitor during a short period of time, e.g. once a day for one or more days either consecutively, intermittently or a combination thereof per week (7 day period). For example, a dose of 300 mg/m^2 per day can be administered for 5 consecutive days for a total of 1500 mg/m^2 per treatment. In another dosing regimen, the number of consecutive days can also be 5, with treatment lasting for 2 or 3 consecutive weeks for a total of 3000 mg/m^2 and 4500 mg/m^2 total treatment.

Typically, an intravenous formulation may be prepared which contains a concentration of HDAC inhibitor of between about 1.0 mg/mL to about 10 mg/mL, e.g. 2.0 mg/mL, 3.0 mg/mL,

4.0 mg/mL, 5.0 mg/mL, 6.0 mg/mL, 7.0 mg/mL, 8.0 mg/mL, 9.0 mg/mL and 10 mg/mL and administered in amounts to achieve the doses described above. In one example, a sufficient volume of intravenous formulation can be administered to a patient in a day such that the total dose for the day is between about 300 and about 1500 mg/m^2.

5 Subcutaneous formulations can be prepared according to procedures well known in the art at a pH in the range between about 5 and about 12, which include suitable buffers and isotonicity agents, as described below. They can be formulated to deliver a daily dose of HDAC inhibitor in one or more daily subcutaneous administrations, e.g., one, two or three times each day.

10 The HDAC inhibitors can also be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, or course, be continuous rather than intermittent throughout the dosage regime.

15 It is apparent to a person skilled in the art that any one or more of the specific dosages and dosage schedules of the HDAC inhibitors are also applicable to any one or more of the anti-cancer agents to be used in the combination treatment. Moreover, the specific dosage and dosage schedule of the anti-cancer agent can further vary, and the optimal dose, dosing schedule, and route of administration can be determined based upon the specific anti-cancer agent that is
20 being used. Further, the various modes of administration, dosages, and dosing schedules described herein merely set forth specific embodiments and should not be construed as limiting the broad scope of the invention. Any permutations, variations, and combinations of the dosages and dosing schedules are included within the scope of the present invention.

25 **Administration of Anti-Cancer Agents**

Any one or more of the specific dosages and dosage schedules of the HDAC inhibitors is also applicable to any one or more of the anti-cancer agents to be used in the combination treatment.

Moreover, the specific dosage and dosage schedule of the one or more anti-cancer agents
30 can further vary, and the optimal dose, dosing schedule and route of administration will be determined based upon the specific anti-cancer agent that is being used.

Of course, the route of administration of SAHA or any one of the other HDAC inhibitors is independent of the route of administration of the anti-cancer agent. A particular route of administration for SAHA is oral administration. Thus, in accordance with this embodiment, SAHA is administered orally, and the one or more anti-cancer agents can be administered orally, parenterally, intraperitoneally, intravenously, intraarterially, transdermally, sublingually, intramuscularly, rectally, transbuccally, intranasally, liposomally, via inhalation, vaginally, intraoccularly, via local delivery by catheter or stent, subcutaneously, intraadiposally, intraarticularly, intrathecally, or in a slow release dosage form.

In addition, the HDAC inhibitor and one or more anti-cancer agents may be administered by the same mode of administration, i.e. all agents administered orally, by IV, etc. However, it is also within the scope of the present invention to administer the HDAC inhibitor by one mode of administration, e.g. oral, and to administer one or more anti-cancer agents by another mode of administration, e.g. IV, or any other ones of the administration modes described hereinabove.

Commonly used anti-cancer agents and daily dosages usually administered include but are not restricted to:

Antimetabolites:	Methotrexate:	20-40 mg/m^2 i.v.
	Methotrexate:	4-6 mg/m^2 p.o.
	Methotrexate:	12000 mg/m^2 high dose therapy
	6-Mercaptopurine:	100 mg/m^2
	6- Thioguanine:	1-2 x 80 mg/m^2 p.o.
	Pentostatin	4 mg/m^2 i.v.
	Fludarabinphosphate:	25 mg/m^2 i.v.
	Cladribine:	0.14 mg/kg BW i.v.
	5-Fluorouracil	500-2600 mg/m^2 i.v.
	Capecitabine:	1250 mg/m^2 p.o.
	Cytarabin:	200 mg/m^2 i.v.
	Cytarabin:	3000 mg/m^2 i.v. high dose therapy
	Gemcitabine:	800-1250 mg/m^2 i.v.
	Hydroxyurea:	800-4000 mg/m^2 p.o.
	Pemetrexed:	250-500 mg/m^2 i.v.

Antimitotic agents and Plant-derived agents:	Vincristine	1.5-2 mg/m^2 i.v.
	Vinblastine	4-8 mg/m^2 i.v.
	Vindesine	2-3 mg/m^2 i.v.
	Etoposide (VP16)	100-200 mg/m^2 i.v.
	Etoposide (VP16)	100 mg p.o.
	Teniposide (VM26)	20-30 mg/m^2 i.v.
	Paclitaxel (Taxol)	175-250 mg/m^2 i.v.
	Docetaxel (Taxotere)	100-150 mg/m^2 i.v.
Antibiotics:	Actinomycin D	0.6 mg/m2 i.v.
	Daunorubicin	45-6.0 mg/m^2 i.v.
	Doxorubicin	45-60 mg/m^2 i.v.
	Epirubicin	60-80 mg/m^2 i.v.
	Idarubicin	10-12 mg/m^2 i.v.
	Idarubicin	35-50 mg/m^2 p.o.
	Mitoxantron	10-12 mg/m^2 i.v.
	Bleomycin	10-15 mg/m^2 i.v., i.m., s.c.
	Mitomycin C	10-20 mg/^{2} i.v.
	Irinotecan (CPT -11)	350 mg/m^2 i.v.
	Topotecan	1.5 mg/m^2 i.v.
Alkylating Agents:	Mustargen	6 mg/m^2 i.v.
	Estramustinphosphate	150-200 mg/m^2 i.v.
	Estramustinphosphate	480-550 mg/m^2 p.o.
	Melphalan	8-10 mg/m^2 i.v.
	Melphalan	15 mg/m^2 i.v.
	Chlorambucil	3-6 mg/m^2 i.v.
	Prednimustine	40-100 mg/m^2 p.o.
	Cyclophosphamide	750-1200 mg/m^2 i.v.
	Cyclophosphamide	50-100 mg/m^2 p.o.
	Ifosfamide	1500-2000 mg/m^2 i. v.

	Trofosfamide	25-200 mg/m^2 p.o.
	Busulfan	2-6 mg/m^2 p.o.
	Treosulfan	5000-8000 mg/m^2 i.v.
	Treosulfan	750-1500 mg/m^2 p.o.
	Thiotepa	12-16 mg/m^2 i.v.
	Carmustin (BCNU)	100 mg/m^2 i.v.
	Lomustin (CCNU)	100-130 mg/m^2 p.o.
	Nimustin (ACNU)	90-100 mg/m^2 i.v.
	Dacarbazine (OTIC)	100-375 mg/m^2 i.v.
	Procarbazine	100 mg/m^2 p.o.
	Cisplatin	20-120 mg/m^2 i.v.
	Carboplatin	300-400 mg/m^2 i.v.
Hormones, Cytokines and Vitamins:	Interferon-α	2-10 x 10^6 IU/m^2
	Prednisone	40-100 mg/m^2 p.o.
	Dexamethasone	8-24 mg p.o.
	G-CSF	5-20 μg/kg BW s.c.
	all-trans Retinoic Acid	45 mg/m^2
	Interleukin-2	18 x 10^6 IU/m^2
	GM-CSF	250 mg/m^2
	Erythropoietin	150 IU/kg tiw

The dosage regimens utilizing one or more anti-cancer agents described herein (or any pharmaceutically acceptable salts or hydrates of such agents, or any free acids, free bases, or other free forms of such agents) can follow the exemplary dosages herein, including those
5 provided for HDAC inhibitors. The dosage can be selected in accordance with a variety of factors including type, species, age, weight, sex and the type of disease being treated; the severity (i.e., stage) of the disease to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound or salt thereof employed. A dosage regimen can be used, for example, to treat, for example, to prevent, inhibit (fully or partially), or arrest the
10 progress of the disease.

In particular embodiments, a tyrosine kinase inhibitor (e.g., Erlotinib) is administered in a dose from about 25 mg to about 50 mg, about 50 mg to about 100 mg, about 100 mg to about 150 mg, about 150 mg to about 200 mg, about 200 mg to about 250 mg, or about 250 mg to 500 mg. As a specific example, Erlotinib can be administered in a dose of about 25 mg, 50 mg, 100 mg, or 150 mg. In a particular embodiment, Erlotinib is administered once daily at a dose of about 150 mg. In another particular embodiment, Erlotinib is administered once daily at a dose of 100 mg. In another particular embodiment, Erlotinib is administered once daily at a dose of 50 mg. In certain aspects, Erlotinib is administered to patients orally. Specifically, Erlotinib can be co-administered with one or more other anti-cancer agents, e.g., SAHA. As examples, SAHA (e.g., Vorinostat) can be administered at a total daily dose of up to 300 mg, 400 mg, 500 mg, or 600 mg, and Erlotinib can be administered at a total daily dose at a total daily dose of up to 50 mg, 100 mg, or 150 mg. SAHA and/or Erlotinib dosages can be administered continuously or intermittently as described in detail herein.

Combination Administration

In accordance with the invention, HDAC inhibitors and one or more anti-cancer agents can be used in the treatment of a wide variety of cancers, including but not limited to solid tumors (e.g., tumors of the head and neck, lung, breast, colon, prostate, bladder, rectum, brain, gastric tissue, bone, ovary, thyroid, or endometrium), hematological malignancies (e.g., leukemias, lymphomas, myelomas), carcinomas (e.g. bladder carcinoma, renal carcinoma, breast carcinoma, colorectal carcinoma), neuroblastoma, or melanoma. Non-limiting examples of these cancers include diffuse large B-cell lymphoma (DLBCL), T-cell lymphomas or leukemias, e.g., cutaneous T-cell lymphoma (CTCL), noncutaneous peripheral T-cell lymphoma, lymphoma associated with human T-cell lymphotrophic virus (HTLV), adult T-cell leukemia/lymphoma (ATLL), as well as acute lymphocytic leukemia, acute nonlymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, myeloma, multiple myeloma, mesothelioma, childhood solid tumors, brain neuroblastoma, retinoblastoma, glioma, Wilms' tumor, bone cancer and soft-tissue sarcomas, common solid tumors of adults such as head and neck cancers (e.g., oral, laryngeal and esophageal), genitourinary cancers (e.g., prostate, bladder, renal, uterine, ovarian, testicular, rectal, and colon), lung cancer (e.g., small cell carcinoma and non-small cell lung carcinoma,

including squamous cell carcinoma and adenocarcinoma), breast cancer, pancreatic cancer, melanoma and other skin cancers, basal cell carcinoma, metastatic skin carcinoma, squamous cell carcinoma of both ulcerating and papillary type, stomach cancer, brain cancer, liver cancer, adrenal cancer, kidney cancer, thyroid cancer, medullary carcinoma, osteosarcoma, soft-tissue
5 sarcoma, Ewing's sarcoma, veticulum cell sarcoma, and Kaposi's sarcoma. Also included are pediatric forms of any of the cancers described herein.

Cutaneous T-cell lymphomas and peripheral T-cell lymphomas are forms of non-Hodgkin's lymphoma. Cutaneous T-cell lymphomas are a group of lymphoproliferative disorders characterized by localization of malignant T lymphocytes to the skin at presentation.
10 CTCL frequently involves the skin, bloodstream, regional lymph nodes, and spleen. Mycosis fungoides (MF), the most common and indolent form of CTCL, is characterized by patches, plaques or tumors containing epidermotropic $CD4^{+}CD45RO^{+}$ helper/memory T cells. MF may evolve into a leukemic variant, Sézary syndrome (SS), or transform to large cell lymphoma. The condition causes severe skin itching, pain and edema. Currently, CTCL is treated topically with
15 steroids, photochemotherapy and chemotherapy, as well as radiotherapy. Peripheral T-cell lymphomas originate from mature or peripheral (not central or thymic) T-cell lymphocytes as a clonal proliferation from a single T-cell and are usually either predominantly nodal or extranodal tumors. They have T-cell lymphocyte cell-surface markers and clonal arrangements of the T-cell receptor genes.

20 Approximately 16,000 to 20,000 people in the U.S. are affected by either CTCL or PTCL. These diseases are highly symptomatic. Patches, plaques and tumors are the clinical names of the different presentations. Patches are usually flat, possibly scaly and look like a "rash." Mycosis fungoides patches are often mistaken for eczema, psoriasis or non-specific dermatitis until a proper diagnosis of mycosis fungoides is made. Plaques are thicker, raised
25 lesions. Tumors are raised "bumps" which may or may not ulcerate. A common characteristic is itching or pruritus, although many patients do not experience itching. It is possible to have one or all three of these phases. For most patients, existing treatments are palliative but not curative.

Lung cancer remains the leading cause of cancer-related mortality in the United States and 30% to 40% of newly diagnosed patients with non-small cell lung cancer present with
30 regionally advanced and unresectable stage III disease (Jemal A *et al. CA Cancer J. Clin.* 2004;54:8-29; Dubey and Schiller *The Oncologist* 2005; 10:282-291; Socinski MA *Semin Oncol.*

2005 32(2 Suppl 3):S114-8). The median survival time of patients with stage IV disease treated with standard chemotherapy regimens is approximately 8-11 months (Schiller JH *et al. N. Engl. J. Med.* 2002;346:92-98; Fossella F *et al. J. Clin. Oncol.* 2003;21:3016-3024). In the relapsed setting, the median survival time with single-agent therapy is approximately 5-7 months, and
5 time to progression is merely 8-10 weeks (Shepherd FA *et al. J. Clin. Oncol.* 2000;18:2095–2103; Fossella FV *et al. J. Clin. Oncol.* 2000;18:2354-2362).

Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer cases. The majority of patients with NSCLC present with advanced disease, and this aggressive tumor is associated with a poor prognosis. The 5-year survival rate for patients with advanced
10 (stage IIIB/IV) NSCLC is < 5% (Ginsberg RJ *et al.* In: *Cancer: Principles and Practice of Oncology*, DeVita VT Jr, Hellman S, Rosenberg SA, eds., 6th Edition, Philadelphia: Lippincott Williams and Wilkins, 2001:925-983). Treatment for NSCLC has been palliative, with the goals of improving symptoms and prolonging survival. Currently, platinum-based regimens are the standard of care for patients with advanced NSCLC (reviewed in Stewart DJ *Oncologist* 2004;9
15 Suppl 6:43-52). Yet, these regimens are associated with severe and often cumulative hematologic and nonhematologic toxicities, limiting dose intensity. Therefore, novel treatments and combination regimens are needed to improve the outcome for these patients.

According to the National Cancer Institute, head and neck cancers account for three percent of all cancers in the U.S. Most head and neck cancers originate in the squamous cells
20 lining the structures found in the head and neck, and are often referred to as squamous cell carcinomas of the head and neck (SCCHN). Some head and neck cancers originate in other types of cells, such as glandular cells. Head and neck cancers that originate in glandular cells are called adenocarcinomas. Head and neck cancers are further defined by the area in which they begin, such as the oral cavity, nasal cavity, larynx, pharynx, salivary glands, and lymph nodes of
25 the upper part of the neck. It is estimated that 38,000 people in the U.S. developed head and neck cancer 2002. Approximately 60% of patients present with locally advanced disease. Only 30% of these patients achieve long-term remission after treatment with surgery and/or radiation. For patients with recurrent and/or metastatic disease, the median survival is approximately six months.

30 Alkylating agents suitable for use in the present invention include but are not limited to bischloroethylamines (nitrogen mustards, e.g., Chlorambucil, Cyclophosphamide, Ifosfamide,

Mechlorethamine, Melphalan, uracil mustard), aziridines (e.g., Thiotepa), alkyl alkone sulfonates (e.g., Busulfan), nitrosoureas (e.g., Carmustine, Lomustine, Streptozocin), nonclassic alkylating agents (e.g., Altretamine, Dacarbazine, and Procarbazine), platinum compounds (e.g., Carboplastin and Cisplatin).

5 Antibiotic agents suitable for use in the present invention are anthracyclines (e.g., Doxorubicin, Daunorubicin, Epirubicin, Idarubicin, and Anthracenedione), Mitomycin C, Bleomycin, Dactinomycin, Plicatomycin.

Antimetabolic agents suitable for use in the present invention include but are not limited to Floxuridine, Fluorouracil, Methotrexate, Leucovorin, Hydroxyurea, Thioguanine,
10 Mercaptopurine, Cytarabine, Pentostatin, Fludarabine Phosphate, Cladribine, Asparaginase, and Gemcitabine. In a particular embodiment, the antimetabolic agent in Gemcitabine.

Hormonal agents suitable for use in the present invention, include but are not limited to, an estrogen, a progestogen, an antiesterogen, an androgen, an antiandrogen, an LHRH analogue, an aromatase inhibitor, Diethylstibestrol, Tamoxifen, Toremifene, Fluoxymesterol, Raloxifene,
15 Bicalutamide, Nilutamide, Flutamide, Aminoglutethimide, Tetrazole, Ketoconazole, Goserelin Acetate, Leuprolide, Megestrol Acetate, and Mifepristone.

Plant-derived agents suitable for use in the present invention include, but are not limited to Vincristine, Vinblastine, Vindesine, Vinzolidine, Vinorelbine, Etoposide, Teniposide, Paclitaxel, and Docetaxel.

20 Biologic agents suitable for use in the present invention include, but are not limited to immuno-modulating proteins, monoclonal antibodies against tumor antigens, tumor suppressor genes, and cancer vaccines. For example, the immuno-modulating protein can be interleukin 2, interleukin 4, interleukin 12, interferon El interferon D, interferon alpha, erythropoietin, granulocyte-CSF, granulocyte, macrophage-CSF, bacillus Calmette-Guerin, Levamisole, or
25 Octreotide. Furthermore, the tumor suppressor gene can be DPC-4, NF-1, NF-2, RB, p53, WTl, BRCA, or BRCA2.

In various aspects of the invention, the treatment procedures are performed sequentially in any order, simultaneously, or a combination thereof. For example, the first treatment procedure, e.g., administration of an HDAC inhibitor, can take place prior to the second treatment
30 procedure, e.g., a second anti-cancer agent, such as a tyrosine kinase inhibitor like Erlotinib and prior to the optional third treatment procedure, e.g., a third anti-cancer agent, after the second

treatment with the second anticancer agent, after the optional third treatment with the third anti-cancer agent, at the same time as the second treatment with the second anticancer agent, at the same time as the optional third treatment with the third anti-cancer agent, or a combination thereof.

5 In one aspect of the invention, a total treatment period can be decided for the HDAC inhibitor. The one or more anti-cancer agents can be administered prior to onset of treatment with the HDAC inhibitor or following treatment with the HDAC inhibitor. In addition, the one or more anti-cancer agents can be administered during the period of HDAC inhibitor administration but does not need to occur over the entire HDAC inhibitor treatment period.

10 Similarly, the HDAC inhibitor can be administered prior to onset of treatment with the one or more anti-cancer agents or following treatment with the one or more anti-cancer agents. In addition, the HDAC inhibitor can be administered during the period of administration of one or more anti-cancer agent but does not need to occur over the entire anti-cancer agent treatment period. Alternatively, the treatment regimen includes pre-treatment with one agent, either the

15 HDAC inhibitor or the one or more anti-cancer agents, followed by the addition of the other agent(s) for the duration of the treatment period.

In a particular embodiment, the combination of the HDAC inhibitor and one or more anti-cancer agents is additive, i.e., the combination treatment regimen produces a result that is the additive effect of each constituent when it is administered alone. In accordance with this

20 embodiment, the amount of HDAC inhibitor and the amount of the one or more anti-cancer agents together constitute an effective amount to treat cancer.

In another embodiment, the combination of the HDAC inhibitor and one or more anti-cancer agent is considered therapeutically synergistic when the combination treatment regimen produces a significantly better anticancer result (e.g., cell growth arrest, apoptosis, induction of

25 differentiation, cell death) than the additive effects of each constituent when it is administered alone at a therapeutic dose. Standard statistical analysis can be employed to determine when the results are significantly better. For example, a Mann-Whitney Test or some other generally accepted statistical analysis can be employed.

In one particular embodiment of the present invention, the HDAC inhibitor and the

30 tyrosine kinase inhibitor can be administered in combination with an additional HDAC inhibitor, an alkylating agent, an antibiotic agent, an antimetabolic agent, a hormonal agent, a plant-derived

agent, an anti-angiogenic agent, a differentiation inducing agent, a cell growth arrest inducing agent, an apoptosis inducing agent, a cytotoxic agent, another tyrosine kinase inhibitor, or a biologic agent.

The combination therapy can act through the induction of cancer cell differentiation, cell growth arrest, and/or apoptosis. The combination of therapy is particularly advantageous, since the dosage of each agent in a combination therapy can be reduced as compared to monotherapy with the agent, while still achieving an overall anti-tumor effect.

Pharmaceutical Compositions

As described above, the compositions comprising the HDAC inhibitor and the one or more anti-cancer agents can be formulated in any dosage form suitable for oral, parenteral, intraperitoneal, intravenous, intraarterial, transdermal, sublingual, intramuscular, rectal, transbuccal, intranasal, liposomal, via inhalation, vaginal, or intraocular administration, for administration via local delivery by catheter or stent, or for subcutaneous, intraadiposal, intraarticular, intrathecal administration, or for administration in a slow release dosage form.

The HDAC inhibitor and the one or more anti-cancer agents can be formulated in the same formulation for simultaneous administration, or they can be in two separate dosage forms, which may be administered simultaneously or sequentially as described above.

The invention also encompasses pharmaceutical compositions comprising pharmaceutically acceptable salts of the HDAC inhibitors and the one or more anti-cancer agents.

Suitable pharmaceutically acceptable salts of the compounds described herein and suitable for use in the method of the invention, are conventional non-toxic salts and can include a salt with a base or an acid addition salt such as a salt with an inorganic base, for example, an alkali metal salt (e.g., lithium salt, sodium salt, potassium salt, etc.), an alkaline earth metal salt (e.g., calcium salt, magnesium salt, etc.), an ammonium salt; a salt with an organic base, for example, an organic amine salt (e.g., triethylamine salt, pyridine salt, picoline salt, ethanolamine salt, triethanolamine salt, dicyclohexylamine salt, N,N'-dibenzylethylenediamine salt, etc.) etc.; an inorganic acid addition salt (e.g., hydrochloride, hydrobromide, sulfate, phosphate, etc.); an organic carboxylic or sulfonic acid addition salt (e.g., formate, acetate, trifluoroacetate, maleate,

tartrate, methanesulfonate, benzenesulfonate, p-toluenesulfonate, etc.); a salt with a basic or acidic amino acid (e.g., arginine, aspartic acid, glutamic acid, etc.) and the like.

The invention also encompasses pharmaceutical compositions comprising hydrates of the HDAC inhibitors and the one or more anti-cancer agents.

5 In addition, this invention also encompasses pharmaceutical compositions comprising any solid or liquid physical form of SAHA or any of the other HDAC inhibitors. For example, The HDAC inhibitors can be in a crystalline form, in amorphous form, and have any particle size. The HDAC inhibitor particles may be micronized, or may be agglomerated, particulate granules, powders, oils, oily suspensions or any other form of solid or liquid physical form.

10 For oral administration, the pharmaceutical compositions can be liquid or solid. Suitable solid oral formulations include tablets, capsules, pills, granules, pellets, and the like. Suitable liquid oral formulations include solutions, suspensions, dispersions, emulsions, oils, and the like.

Any inert excipient that is commonly used as a carrier or diluent may be used in the formulations of the present invention, such as for example, a gum, a starch, a sugar, a cellulosic

15 material, an acrylate, or mixtures thereof. The compositions may further comprise a disintegrating agent and a lubricant, and in addition may comprise one or more additives selected from a binder, a buffer, a protease inhibitor, a surfactant, a solubilizing agent, a plasticizer, an emulsifier, a stabilizing agent, a viscosity increasing agent, a sweetener, a film forming agent, or any combination thereof. Furthermore, the compositions of the present invention may be in the

20 form of controlled release or immediate release formulations.

The HDAC inhibitors can be administered as active ingredients in admixture with suitable pharmaceutical diluents, excipients or carriers (collectively referred to herein as "carrier" materials or "pharmaceutically acceptable carriers") suitably selected with respect to the intended form of administration. As used herein, "pharmaceutically acceptable carrier" is

25 intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference.

30 For liquid formulations, pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, emulsions or oils. Examples of non-aqueous solvents are

propylene glycol, polyethylene glycol, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions, or suspensions, including saline and buffered media. Examples of oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, mineral oil, olive oil, sunflower oil, and fish-liver oil. Solutions or suspensions can also include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide.

Liposomes and non-aqueous vehicles such as fixed oils may also be used. 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 active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Solid carriers/diluents include, but are not limited to, a gum, a starch (e.g., corn starch, pregelatinized starch), a sugar (e.g., lactose, mannitol, sucrose, dextrose), a cellulosic material (e.g., microcrystalline cellulose), an acrylate (e.g., polymethylacrylate), calcium carbonate, magnesium oxide, talc, or mixtures thereof.

In addition, the compositions may further comprise binders (e.g., acacia, cornstarch, gelatin, carbomer, ethyl cellulose, guar gum, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, povidone), disintegrating agents (e.g., cornstarch, potato starch, alginic acid, silicon dioxide, croscarmellose sodium, crospovidone, guar gum, sodium starch glycolate, Primogel), buffers (e.g., tris-HCI, acetate, phosphate) of various pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts), protease inhibitors, surfactants (e.g., sodium lauryl sulfate), permeation enhancers, solubilizing agents (e.g., glycerol, polyethylene glycerol), a glidant (e.g., colloidal silicon dioxide), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite, butylated hydroxyanisole), stabilizers (e.g., hydroxypropyl cellulose, hydroxypropylmethyl cellulose), viscosity increasing agents (e.g., carbomer, colloidal silicon dioxide, ethyl cellulose, guar gum),

sweeteners (e.g., sucrose, aspartame, citric acid), flavoring agents (e.g., peppermint, methyl salicylate, or orange flavoring), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), lubricants (e.g., stearic acid, magnesium stearate, polyethylene glycol, sodium lauryl sulfate), flow-aids (e.g., colloidal silicon dioxide), plasticizers (e.g., diethyl phthalate, triethyl citrate), emulsifiers (e.g., carbomer, hydroxypropyl cellulose, sodium lauryl sulfate), polymer coatings (e.g., poloxamers or poloxamines), coating and film forming agents (e.g., ethyl cellulose, acrylates, polymethacrylates) and/or adjuvants.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

The preparation of pharmaceutical compositions that contain an active component is well understood in the art, for example, by mixing, granulating, or tablet-forming processes. The active therapeutic ingredient is often mixed with excipients that are pharmaceutically acceptable and compatible with the active ingredient. For oral administration, the active agents are mixed

with additives customary for this purpose, such as vehicles, stabilizers, or inert diluents, and converted by customary methods into suitable forms for administration, such as tablets, coated tablets, hard or soft gelatin capsules, aqueous, alcoholic, or oily solutions and the like as detailed above.

5 The amount of the compound administered to the patient is less than an amount that would cause toxicity in the patient. In the certain embodiments, the amount of the compound that is administered to the patient is less than the amount that causes a concentration of the compound in the patient's plasma to equal or exceed the toxic level of the compound. In particular embodiments, the concentration of the compound in the patient's plasma is maintained
10 at about 10 nM. In another embodiment, the concentration of the compound in the patient's plasma is maintained at about 25 nM. In another embodiment, the concentration of the compound in the patient's plasma is maintained at about 50 nM. In another embodiment, the concentration of the compound in the patient's plasma is maintained at about 100 nM. In another embodiment, the concentration of the compound in the patient's plasma is maintained at about
15 500 nM. In another embodiment, the concentration of the compound in the patient's plasma is maintained at about 1,000 nM. In another embodiment, the concentration of the compound in the patient's plasma is maintained at about 2,500 nM. In another embodiment, the concentration of the compound in the patient's plasma is maintained at about 5,000 nM. The optimal amount of the compound that should be administered to the patient in the practice of the present
20 invention will depend on the particular compound used and the type of cancer being treated.

The percentage of the active ingredient and various excipients in the formulations may vary. For example, the composition may comprise between 20 and 90%, or specifically between 50-70% by weight of the active agent.

For IV administration, Glucuronic acid, L-lactic acid, acetic acid, citric acid or any
25 pharmaceutically acceptable acid/conjugate base with reasonable buffering capacity in the pH range acceptable for intravenous administration can be used as buffers. Sodium chloride solution wherein the pH has been adjusted to the desired range with either acid or base, for example, hydrochloric acid or sodium hydroxide, can also be employed. Typically, a pH range for the intravenous formulation can be in the range of from about 5 to about 12. A particular pH range
30 for intravenous formulation comprising an HDAC inhibitor, wherein the HDAC inhibitor has a hydroxamic acid moiety, can be about 9 to about 12.

Subcutaneous formulations can be prepared according to procedures well known in the art at a pH in the range between about 5 and about 12, which include suitable buffers and isotonicity agents. They can be formulated to deliver a daily dose of the active agent in one or more daily subcutaneous administrations. The choice of appropriate buffer and pH of a formulation, depending on solubility of the HDAC inhibitor to be administered, is readily made by a person having ordinary skill in the art. Sodium chloride solution wherein the pH has been adjusted to the desired range with either acid or base, for example, hydrochloric acid or sodium hydroxide, can also be employed in the subcutaneous formulation. Typically, a pH range for the subcutaneous formulation can be in the range of from about 5 to about 12. A particular pH range for subcutaneous formulation of an HDAC inhibitor a hydroxamic acid moiety can be about 9 to about 12.

The compositions of the present invention can also be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, or course, be continuous rather than intermittent throughout the dosage regime.

The present invention also provides in-vitro methods for selectively inducing terminal differentiation, cell growth arrest and/or apoptosis of neoplastic cells, thereby inhibiting proliferation of such cells, by contacting the cells with a first amount of suberoylanilide hydroxamic acid (SAHA) or a pharmaceutically acceptable salt or hydrate thereof, a second amount of Erlotinib or a pharmaceutically acceptable salt or hydrate thereof, and optionally a third amount of an anti-cancer agent or a pharmaceutically acceptable salt or hydrate thereof, wherein the first, second, and optional third amounts together comprise an amount effective to induce terminal differentiation, cell growth arrest of apoptosis of the cells.

Although the methods of the present invention can be practiced *in vitro*, it is contemplated that a particular embodiment for the methods of selectively inducing terminal differentiation, cell growth arrest and/or apoptosis of neoplastic cells will comprise contacting the cells *in vivo*, i.e., by administering the compounds to a subject harboring neoplastic cells or tumor cells in need of treatment.

As such, the present invention also provides methods for selectively inducing terminal differentiation, cell growth arrest and/or apoptosis of neoplastic cells, thereby inhibiting

proliferation of such cells in a subject by administering to the subject a first amount of suberoylanilide hydroxamic acid (SAHA) or a pharmaceutically acceptable salt or hydrate thereof, in a first treatment procedure, a second amount of Erlotinib or a pharmaceutically acceptable salt or hydrate thereof, in a second treatment procedure, and optionally a third amount of an anti-cancer agent or a pharmaceutically acceptable salt or hydrate thereof, in a third treatment procedure, wherein the first, second, and optional third amounts together comprise an amount effective to induce terminal differentiation, cell growth arrest of apoptosis of the cells.

The invention is illustrated in the examples that follow. This section is set forth to aid in an understanding of the invention but is not intended to, and should not be construed to limit in any way the invention as set forth in the claims which follow thereafter.

EXAMPLES

The examples are presented in order to more fully illustrate the various embodiments of the invention. These examples should in no way be construed as limiting the scope of the invention recited in the appended claims.

EXAMPLE 1: Synthesis of SAHA

SAHA can be synthesized according to the method outlined below, or according to the method set forth in US Patent 5,369,108, the contents of which are incorporated by reference in their entirety, or according to any other method.

Synthesis of SAHA

Step 1 – Synthesis of Suberanilic acid

$$HO\overset{O}{\overset{\|}{C}}-(CH_2)_6-\overset{O}{\overset{\|}{C}}OH \;+\; C_6H_5NH_2 \longrightarrow C_6H_5-\overset{H}{\overset{|}{N}}-\overset{O}{\overset{\|}{C}}-(CH_2)_6-\overset{O}{\overset{\|}{C}}-OH$$

In a 22 L flask was placed 3,500 g (20.09 moles) of suberic acid, and the acid melted with heat. The temperature was raised to 175°C, and then 2,040 g (21.92 moles) of aniline was added. The temperature was raised to 190°C and held at that temperature for 20 minutes. The

melt was poured into a Nalgene tank that contained 4,017 g of potassium hydroxide dissolved in 50 L of water. The mixture was stirred for 20 minutes following the addition of the melt. The reaction was repeated at the same scale, and the second melt was poured into the same solution of potassium hydroxide. After the mixture was thoroughly stirred, the stirrer was turned off, and 5 the mixture was allowed to settle.

The mixture was then filtered through a pad of Celite (4,200 g). The product was filtered to remove the neutral by-product from attack by aniline on both ends of suberic acid. The filtrate contained the salt of the product, and also the salt of unreacted suberic acid. The mixture was allowed to settle because the filtration was very slow, taking several days. The filtrate was 10 acidified using 5 L of concentrated hydrochloric acid; the mixture was stirred for one hour, and then allowed to settle overnight. The product was collected by filtration, and washed on the funnel with deionized water (4 x 5 L). The wet filter cake was placed in a 72 L flask with 44 L of deionized water, the mixture heated to 50°C, and the solid isolated by a hot filtration (the desired product was contaminated with suberic acid which is has a much greater solubility in hot 15 water. Several hot triturations were done to remove suberic acid. The product was checked by NMR [D_6DMSO] to monitor the removal of suberic acid). The hot trituration was repeated with 44 L of water at 50°C. The product was again isolated by filtration, and rinsed with 4 L of hot water. It was dried over the weekend in a vacuum oven at 65°C using a Nash pump as the vacuum source (the Nash pump is a liquid ring pump (water) and pulls a vacuum of about 29 20 inch of mercury. An intermittent argon purge was used to help carry off water); 4,182.8 g of suberanilic acid was obtained.

The product still contained a small amount of suberic acid; therefore the hot trituration was done portionwise at 65°C, using about 300 g of product at a time. Each portion was filtered, and rinsed thoroughly with additional hot water (a total of about 6 L). This was repeated to 25 purify the entire batch. This completely removed suberic acid from the product. The solid product was combined in a flask and stirred with 6 L of methanol/water (1:2), and then isolated by filtration and air dried on the filter over the week end. It was placed in trays and dried in a vacuum oven at 65°C for 45 hours using the Nash pump and an argon bleed. The final product has a weight of 3,278.4 g (32.7% yield).

Step 2 –Synthesis of Methyl Suberanilate

$$C_6H_5-NH-C(=O)-(CH_2)_6-C(=O)-OH \longrightarrow C_6H_5-NH-C(=O)-(CH_2)_6-C(=O)-OCH_3$$

To a 50 L flask fitted with a mechanical stirrer, and condenser was placed 3,229 g of suberanilic acid from the previous step, 20 L of methanol, and 398.7 g of Dowex 50WX2-400 resin. The mixture was heated to reflux and held at reflux for 18 hours. The mixture was filtered to remove the resin beads, and the filtrate was taken to a residue on a rotary evaporator.

The residue from the rotary evaporator was transferred into a 50 L flask fitted with a condenser and mechanical stirrer. To the flask was added 6 L of methanol, and the mixture heated to give a solution. Then 2 L of deionized water was added, and the heat turned off. The stirred mixture was allowed to cool, and then the flask was placed in an ice bath, and the mixture cooled. The solid product was isolated by filtration, and the filter cake was rinsed with 4 L of cold methanol/water (1:1). The product was dried at 45°C in a vacuum oven using a Nash pump for a total of 64 hours to give 2,850.2 g (84% yield) of methyl suberanilate.

Step 3 – Synthesis of Crude SAHA

$$C_6H_5-NH-C(=O)-(CH_2)_6-C(=O)-OCH_3 + NH_2OH \cdot HCl \longrightarrow C_6H_5-NH-C(=O)-(CH_2)_6-C(=O)-NH-OH$$

To a 50 L flask with a mechanical stirrer, thermocouple, and inlet for inert atmosphere was added 1,451.9 g of hydroxylamine hydrochloride, 19 L of anhydrous methanol, and a 3.93 L of a 30% sodium methoxide solution in methanol. The flask was then charged with 2,748.0 g of methyl suberanilate, followed by 1.9 L of a 30% sodium methoxide solution in methanol. The mixture was allowed to stir for 16 hr and 10 minutes. Approximately one half of the reaction mixture was transferred from the reaction flask (flask 1) to a 50 L flask (flask 2) fitted with a mechanical stirrer. Then 27 L of deionized water was added to flask 1 and the mixture was

stirrer for 10 minutes. The pH was taken using a pH meter; the pH was 11.56. The pH of the mixture was adjusted to 12.02 by the addition of 100 ml of the 30% sodium methoxide solution in methanol; this gave a clear solution (the reaction mixture at this time contained a small amount of solid. The pH was adjusted to give a clear solution from which the precipitation the
5 product would be precipitated). The reaction mixture in flask 2 was diluted in the same manner; 27 L of deionized water was added, and the pH adjusted by the addition of 100 ml of a 30 % sodium methoxide solution to the mixture, to give a pH of 12.01 (clear solution).

The reaction mixture in each flask was acidified by the addition of glacial acetic acid to precipitate the product. Flask 1 had a final pH of 8.98, and Flask 2 had a final pH of 8.70. The
10 product from both flasks was isolated by filtration using a Buchner funnel and filter cloth. The filter cake was washed with 15 L of deionized water, and the funnel was covered and the product was partially dried on the funnel under vacuum for 15.5 hr. The product was removed and placed into five glass trays. The trays were placed in a vacuum oven and the product was dried to constant weight. The first drying period was for 22 hours at 60°C using a Nash pump as the
15 vacuum source with an argon bleed. The trays were removed from the vacuum oven and weighed. The trays were returned to the oven and the product dried for an additional 4 hr and 10 minutes using an oil pump as the vacuum source and with no argon bleed. The material was packaged in double 4-mill polyethylene bags, and placed in a plastic outer container. The final weight after sampling was 2633.4 g (95.6%).

20

Step 4 – Recrystallization of Crude SAHA

The crude SAHA was recrystallized from methanol/water. A 50 L flask with a mechanical stirrer, thermocouple, condenser, and inlet for inert atmosphere was charged with the crude SAHA to be crystallized (2,525.7 g), followed by 2,625 ml of deionized water and 15,755
25 ml of methanol. The material was heated to reflux to give a solution. Then 5,250 ml of deionized water was added to the reaction mixture. The heat was turned off, and the mixture was allowed to cool. When the mixture had cooled sufficiently so that the flask could be safely handled (28°C), the flask was removed from the heating mantle, and placed in a tub for use as a cooling bath. Ice/water was added to the tub to cool the mixture to -5°C. The mixture was held
30 below that temperature for 2 hours. The product was isolated by filtration, and the filter cake washed with 1.5 L of cold methanol/water (2:1). The funnel was covered, and the product was

partially dried under vacuum for 1.75 hr. The product was removed from the funnel and placed in 6 glass trays. The trays were placed in a vacuum oven, and the product was dried for 64.75 hr at 60°C using a Nash pump as the vacuum source, and using an argon bleed. The trays were removed for weighing, and then returned to the oven and dried for an additional 4 hours at 60°C
5 to give a constant weight. The vacuum source for the second drying period was an oil pump, and no argon bleed was used. The material was packaged in double 4-mill polyethylene bags, and placed in a plastic outer container. The final weight after sampling was 2,540.9 g (92.5%).

In other experiments, crude SAHA was crystallized using the following conditions:

10 Table 1: SAHA Crystallization Conditions

Solvent	Water	Agitation	Time (hr)
Methanol	-	Off	2
Methanol	-	On	72
Ethanol	-	On	72
Isopropanol	-	Off	72
Ethanol	15%	On	2
Methanol	15%	Off	72
Ethanol	15%	Off	72
Ethanol	15%	On	72
Methanol	15%	On	72

All these reaction conditions produced SAHA Polymorph I.

EXAMPLE 2: Generation of Wet-Milled Small Particles in 1:1 Ethanol/Water

15 The SAHA Polymorph I crystals were suspended in 1:1 (by volume) EtOH/water solvent mixture at a slurry concentration ranging from 50 mg/gram to 150 mg/gram (crystal/solvent mixture). The slurry was wet milled with IKA-Works Rotor-Stator high shear homogenizer model T50 with superfine blades at 20-30 m/s, until the mean particle size of SAHA was less than 50 μm and 95% less than 100 μm, while maintaining the temperature at room temperature.
20 The wet-milled slurry was filtered and washed with the 1:1 EtOH/water solvent mixture at room

temperature. The wet cake was then dried at 40°C. The final mean particle size of the wet-milled material was less than 50 μm as measured by the Microtrac method below.

Particle size was analyzed using an SRA-150 laser diffraction particle size analyzer, manufactured by Microtrac Inc. The analyzer was equipped with an ASVR (Automatic Small Volume Recirculator). 0.25 wt% lecithin in ISOPAR G was used as the dispersing fluid. Three runs were recorded for each sample and an average distribution was calculated. Particle size distribution (PSD) was analyzed as a volume distribution. The mean particle size and 95% < values based on volume were reported.

EXAMPLE 2A: Large Scale Generation of Wet-Milled Small Particles in 1:1 Ethanol/Water

56.4 kg SAHA Polymorph I crystals were charged to 610 kg (10.8 kg solvent per kg SAHA) of a 50% vol/vol solution of 200 proof punctilious ethanol and water (50/50 EtOH/Water) at 20-25°C. The slurry (~ 700 L) was recirculated through an IKA Works wet-mill set with super-fine generators until reaching a steady-state particle size distribution. The conditions were: DR3-6, 23 m/s rotor tip speed, 30-35 Lpm, 3 gen, ~ 96 turnovers (a turnover is one batch volume passed through one gen), ~ 12 hrs.

$$Approx.\ Mill\ Time(hr) = \frac{96 \times Batch\ Volume\ (L)}{Natural\ Draft\ of\ Mill\ (Lpm) \times \#\ of\ Generators \times 60}$$

The wet cake was filtered, washed 2X with water (total 6 kg/kg, ~ 340 kg) and vacuum dried at 40-45°C. The dry cake was then sieved (595 μm screen) and packed as Fine API.

EXAMPLE 3: Growth of Large Crystals of Mean Particle Size 150 μm in 1:1 Ethanol/Water

25 grams of SAHA Polymorph I crystals and 388 grams of 1:1 Ethanol/water solvent mixture were charged into a 500 ml jacketed resin kettle with a glass agitator. The slurry was wet milled to a particle size less than 50 μm at room temperature following the steps of Example 2. The wet-milled slurry was heated to 65°C to dissolve ~ 85% of the solid. The heated slurry was aged at 65°C for 1-3 hours to establish a ~ 15 % seed bed. The slurry was mixed in the resin kettle under 20 psig pressure, and at an agitator speed range of 400-700 rpm.

The batch was then cooled slowly to 5°C: 65 to 55°C in 10 hours, 55 to 45°C in 10 hours, 45 to 5°C in 8 hours. The cooled batch was aged at 5°C for one hour to reach a target supernatant concentration of less than 5 mg/g, in particular, 3 mg/g. The batch slurry was filtered and washed with 1:1 EtOH/water solvent mixture at 5°C. The wet cake was dried at 40°C under
5 vacuum. The dry cake had a final particle size of ~ 150 μm with 95% particle size < 300 μm according to the Microtrac method.

EXAMPLE 4: Growth of Large Crystals with Mean Particle Size of 140 μm in 1:1 Ethanol/Water

10 7.5 grams of SAHA Polymorph I crystals and 70.7 grams of 1:1 EtOH/water solvent mixture were charged into a seed preparation vessel (500-ml jacketed resin kettle). The seed slurry was wet milled to a particle size less than 50 μm at room temperature following the steps of Example 2 above. The seed slurry was heated to 63-67°C and aged over 30 minutes to 2 hours.

15 In a separate crystallizer (1-liter jacketed resin kettle), 17.5 grams of SAHA Polymorph I crystals and 317.3 grams of 1:1 EtOH/water solvent mixture were charged. The crystallizer was heated to 67-70°C to dissolve all solid SAHA crystals first, and then was cooled to 60-65°C to keep a slightly supersaturated solution.

The seed slurry from the seed preparation vessel was transferred to the crystallizer. The
20 slurry was mixed in the resin kettle under 20 psig pressure, and at an agitator speed range similar to that in Example 3. The batch slurry was cooled slowly to 5°C according to the cooling profile in Example 3. The batch slurry was filtered and washed with 1:1 EtOH/water solvent mixture at 5°C. The wet cake was dried at 40°C under vacuum. The dry cake had a final particle size of about 140 μm with 95% particle size < 280 μm.

25

EXAMPLE 4A: Large Scale Growth of Large Crystals in 1:1 Ethanol/Water

21.9 kg of the Fine API dry cake from Example 2A (30% of total) and 201 kg of 50/50 EtOH/Water solution (2.75 kg solvent/kg total SAHA) was charged to Vessel #1 – the Seed Preparation Tank. 51.1 kg of SAHA Polymorph I crystals (70% of total) and 932 kg 50/50
30 EtOH/Water (12.77 kg solvent/kg total SAHA) was charged to Vessel #2 – the Crystallizer. The Crystallizer was pressurized to 20-25 psig and the contents heated to 67-70°C while maintaining

the pressure to fully dissolve the crystalline SAHA. The contents were then cooled to 61-63°C to supersaturate the solution. During the aging process in the Crystallizer, the Seed Prep Tank was pressurized to 20-25 psig, the seed slurry was heated to 64°C (range: 62-66°C), aged for 30 minutes while maintaining the pressure to dissolve ~ ½ of the seed solids, and then cooled to 61-63°C.

The hot seed slurry was rapidly transferred from the Seed Prep Tank to the Crystallizer (no flush) while maintaining both vessel temperatures. The nitrogen pressure in the Crystallizer was re-established to 20-25 psig and the batch was aged for 2 hours at 61-63°C. The batch was cooled to 5°C in three linear steps over 26 hours: (1) from 62°C to 55°C over 10 hours; (2) from 55°C to 45°C over 6 hours; and (3) from 45°C to 5°C over 10 hours. The batch was aged for 1 hr and then the wet cake was filtered and washed 2X with water (total 6 kg/kg, ~ 440 kg), and vacuum dried at 40-45°C. The dry cake from this recrystallization process is packed-out as the Coarse API. Coarse API and Fine API were blended at a 70/30 ratio.

EXAMPLE 5: Generation of Wet-milled Small Particles Batch 288

SAHA Polymorph I crystals were suspended in ethanolic aqueous solution (100% ethanol to 50% ethanol in water by volume) at a slurry concentration ranging from 50 mg/gram to 150 mg/gram (crystal/solvent mixture). The slurry was wet milled with IKA-Works Rotor-Stator high shear homogenizer model T50 with superfine blades at 20-35 m/s, until the mean particle size of SAHA was less than 50 μm and 95% less than 100 μm, while maintaining the temperature at room temperature. The wet-milled slurry was filtered and washed with EtOH/water solvent mixture at room temperature. The wet cake was then dried at 40°C. The final mean particle size of the wet-milled material was less than 50 μm as measured by the Microtrac method as described before.

EXAMPLE 6: Growth of Large Crystals Batch 283

24 grams of SAHA Polymorph I crystals and 205 ml of 9:1 Ethanol/water solvent mixture were charged into a 500 ml jacketed resin kettle with a glass agitator. The slurry was wet milled to a particle size less than 50 μm at room temperature following the steps of Example 1. The wet-milled slurry was heated to 65°C to dissolve ~ 85% of the solid. The heated slurry

was aged at 64-65°C for 1-3 hours to establish a ~ 15 % seed bed. The slurry was mixed at an agitator speed range of 100 – 300 rpm.

The batch was then cooled to 20°C with one heat-cool cycle: 65°C to 55°C in 2 hours, 55°C for 1 hour, 55°C to 65°C over ~ 30 minutes, age at 65°C for 1 hour, 65°C to 40°C in 5 hours, 40°C to 30°C in 4 hours, 30°C to 20°C over 6 hours. The cooled batch was aged at 20°C for one hour. The batch slurry was filtered and washed with 9:1 EtOH/water solvent mixture at 20°C. The wet cake was dried at 40°C under vacuum. The dry cake had a final particle size of ~ 150 μm with 95% particle size < 300 μm per Microtrac method.

30% of the batch 288 crystals and 70% of the batch 283 crystals were blended to produce capsules containing about 100 mg of suberoylanilide hydroxamic acid; about 44.3 mg of microcrystalline cellulose; about 4.5 mg of croscarmellose sodium; and about 1.2 mg of magnesium stearate.

EXAMPLE 7: Assays for Viability of Non-Small Cell Lung Cancer Cell Lines Treated with SAHA and Erlotinib

On Day 1, 100 μL of non-small cell lung cancer cell lines H460 and A549 were each plated onto white 96 well plates at a density of 4000 cells/well. The outer wells on the plates were not used. On Day 2, a 10X-stock was prepared for the highest concentration of SAHA (Vorinostat) and Erlotinib (Tarceva®) used as single agents and in combination. In particular, a 11 μM SAHA solution and 100 μM Erlotinib was prepared for the H460 cell line. For each compound, 12.5 μL was added to the corresponding wells in duplicate for each treatment concentration. The cells were incubated with the compounds (i.e. SAHA and Erlotinib) for 72 hours.

On Day 5, the Vialight assay (cell proliferation assay, Cambrex Cat# LT07-121) was performed. All reagents were allowed to warm to room temperature before use. AMR PLUS was reconstituted in Assay Buffer. This was left for 15 minutes at room temperature to ensure complete rehydration. One white 96 well plate was removed from the incubator for each cell line. The plate was allowed to cool to room temperature for at least 5 minutes. Next, 50 μl of Cell Lysis Reagent was added to each well and incubated at least 10 minutes. Following this, 100 μl of AMR PLUS was added to each appropriate well. The plate was incubated for 2 minutes at room temperature. The plate was placed in a Victor Spectrophotometer and measured

for luminescence. This produced data for 72 hour cell viability. Results are shown in FIGS. 1A and 1B.

EXAMPLE 8: A Phase I/II Clinical Trial of Oral SAHA in Combination With Erlotinib in Patients With Relapsed/Refractory Non-Small Cell Lung Cancer

This clinical study is used to evaluate the safety, tolerability, pharmacokinetics, and efficacy of SAHA (Vorinostat) administered in combination with Erlotinib (Tarceva®) to patients with advanced non-small cell lung cancer.

Part I: This study is used to determine the maximum tolerated dose (MTD) of SAHA in combination with Erlotinib when administered to patients with relapsed/refractory non-small-cell lung cancer (NSCLC) in 2 different dose escalation regimens. The study is also used to assess the safety and tolerability of these regimens.

Part II: This study is used to evaluate activity, as assessed by objective response rate and progression rate at 8 weeks, in patients treated with SAHA and Erlotinib in combination. The study is also used to assess the pharmacokinetics of SAHA and Erlotinib when administered in combination at the recommended Phase II dose (RP2D). The study is further used to assess the safety and tolerability of these regimens. In addition the study is used to evaluate the effects of SAHA in combination with Erlotinib on time to response, response duration, and progression-free survival.

In Part I, the study looks to determine that the administration of SAHA in combination with Erlotinib to patients with relapsed/refractory NSCLC is sufficiently safe and tolerated to permit further study. In Part II, the study looks to determine that SAHA in combination with Erlotinib has an antitumor effect at the RP2D in patients with relapsed/refractory NSCLC, and is generally safe and tolerable.

Study Design and Duration: This is a multicenter, open-label, randomized dose escalation study in patients with relapsed/refractory non-small-cell lung cancer.

SAHA Dosing Schedule

Dose Level	Cohort A	Cohort B
1	300 mg q.d. 3 out of 7 days	200 mg b.i.d. 3 out of 7 days
2	400 mg q.d. 3 out of 7 days	300 mg b.i.d. 3 out of 7 days

Dose Level	Cohort A	Cohort B
3	500 mg q.d. 3 out of 7 days	300 mg b.i.d. 7 out of 14 days

In Part I of the study, patients are randomized to 1 of 2 SAHA dose escalation regimens (Cohort A or B, above). In both regimens, Erlotinib is administered continuously at a dose of 150 mg by mouth (P.O.) daily. Three patients are entered at each dose level. If none of the first 3 patients at a dose level experience a dose-limiting toxicity (DLT) during the first 28-day treatment cycle (Cycle 1), then 3 new patients may be entered at the next higher dose level. If 1 of 3 patients experience a DLT during Cycle 1, up to 3 more patients will be treated at that same dose level (total n=6). If 2 or more patients experience a DLT during Cycle 1 at a given dose level, no further patients will be treated at that dose. The MTD will be defined as the highest dose level at which <2 of 6 patients experience a DLT. Cohorts A and B enroll concurrently. The principal investigator consults to determine the appropriate dose level for a new patient. A total of 6 patients are enrolled at the presumed recommended phase II dose (RP2D) for each cohort even in the absence of toxicity in the first 3 patients.

In Part II of the study, once the MTD has been established, the RP2D and schedule(s) are determined. Interim analysis is performed following the first 6 target patients enrolled at the RP2D after two cycles of treatment and again following the first 13 target patients enrolled at the RP2D after two cycles of treatment. Patients are continued in treatment with subsequent cycles if they have non-progressive disease and acceptable toxicity. Patients are treated with 2 additional cycles beyond confirmation of a complete response.

Patient Sample: During the Phase I portion of the study, a minimum of 3 and a maximum of 6 patients will be enrolled at each initial dose level to establish the MTD of SAHA administered in combination with Erlotinib. Three dose levels are planned in each treatment arm, and patients are randomized to either cohort. Dose escalation proceeds separately in each cohort. Once randomized to a cohort, the patient is assigned to the appropriate dose level. Once the MTD is established for each regimen, the RP2D is selected, and approximately 60 additional patients are enrolled to allow a more detailed investigation of the safety, efficacy, and pharmacokinetics of SAHA administration.

Dosage/Dosage Form, Route, and Dose Regimen: For Cohort A, SAHA will be administered in repeated 28-day cycles initially at 300 mg once daily (q.d.) for 3 consecutive

days, followed by a 4-day rest period. Barring DLT in Cycle 1 for patients enrolled on Dose Level 1, the SAHA dose will be escalated to 400 mg q.d. for 3 consecutive days, followed by a 4-day rest period and then to 500 mg q.d. for 3 consecutive days, followed by a 4-day rest period. Erlotinib will be administered continuously at a dose of 150 mg P.O. daily for all planned dose 5 levels.

For Cohort B, SAHA will be administered in repeated 28-day cycles initially at 200 mg twice daily (b.i.d.), for 3 consecutive days, followed by a 4-day rest period barring DLT in Cycle 1, the next dose level will be escalated to 300 mg b.i.d. for 3 consecutive days followed by a 4-day rest and then to 300 mg b.i.d. for 7 consecutive days followed by a 7-day rest. Erlotinib is 10 administered continuously at a dose of 150 mg P.O. daily. A minimum of 3 patients must have completed and tolerated a full 28-day cycle of therapy at a given dose level prior to the treatment of patients at the next highest dose level of SAHA. Both treatment arms will be performed in an outpatient setting, and intrapatient dose escalation of SAHA will not be allowed.

In both the Phase I and Phase II components of the study, in the event that an individual 15 patient experiences a DLT other than rash, both the SAHA and Erlotinib will be held until the DLT resolves to Grade 1 intensity or less (or baseline, if higher than Grade 1). DLTs are defined as set by the National Cancer Institute (NCI) Common Terminology for Adverse Events (CTCAE) version 3.0.

If DLT resolution occurs within 2 weeks of holding both drugs, the patient then resumes 20 a modified dose of Erlotinib at 100 mg P.O. q.d., and modified dose #1 of SAHA. After the DLT has been resolved for one cycle, intrapatient dose escalation may occur for Erlotinib by 50 mg increments per week to a final dose of 150 mg P.O. q.d. Following a second DLT other than rash, if DLT resolution occurs within 2 weeks of holding both drugs, the patient then resumes a dose of Erlotinib that has been modified by 50 mg q.d. and modified dose #2 of SAHA. If DLT 25 resolution does not occur within 2 weeks or if the patient requires more than 2 SAHA dose modifications, they are discontinued from the study. Intrapatient Erlotinib dose escalation may occur as noted above. If there is a DLT of rash, dose modifications is made to Erlotinib instead of SAHA, in 50 mg increments. Intrapatient dose escalation of Erlotinib may occur after the patient's DLT resolves and the patient is stable for one cycle. This escalation occurs in 50 mg 30 increments weekly, up to a final dose of 150 mg P.O. q.d. There is no intrapatient SAHA dose escalation. Dose modifications are detailed below.

Efficacy Measurements and Safety Measurements: Disease response/progression is assessed by the investigator using computerized tomography scan (CT) or magnetic resonance imaging (MRI) and standard response criteria in solid tumors (RECIST). At study entry, patients must have at least 1 site of disease, defined as tumor, which can be accurately measured by conventional or spiral CT scan or MRI of the chest through the adrenals, including the liver. The objective response rate, progression rate, time to response, response duration, and progression-free survival for SAHA and Erlotinib used in combination is determined. Investigators monitor disease progression/response every 57 days beginning with Cycle 3, or more frequently if appropriate, and report accordingly. Vital signs, oxygen saturation of the blood, physical examinations, Eastern Cooperative Oncology Group (ECOG) performance status, adverse events (AEs), laboratory safety tests, and electrocardiograms (ECG) are obtained or assessed prior to drug administration and at designated intervals throughout the study.

Data Analysis: The activity of the RP2D obtained in each cohort is compared using an adaptive 3-stage modified multinomial design (Zee B *et al.*, *Journal Biopharm. Statistics*, 1999;9(2):351-63). At Stage 0, a preliminary interim analysis occurs when a total of 6 target population patients, including those from Part I at the same dose, are enrolled and include a minimum of 8 weeks of follow-up. A decision is made as whether to stop the study due to substantial efficacy evidence or to continue both dose schedules to Stage 1. At Stage 1, a total of 13 target patients, including the 6 target population patients from Stage 0, are studied in each schedule. An interim analysis occurs after Stage 1 when all the target patients include a minimum of 8 weeks of follow-up. After this interim analysis, a decision is made as whether to stop the study, to continue one schedule or to continue 2 schedules to Stage 2. For dose schedule(s) chosen to continue, an additional 9 target population patients are studied so that Stage 2 has a total of 22 target population patients for study in each remaining schedule.

If the true response rate and progression rate after Week 8 (Day 57 +3 days) are 20% and 30%, respectively, for the superior dose schedule and 10% and 50%, respectively, for the inferior dose schedule, with the 3-stage design, there is a 71% chance that only the superior one is selected, an 8% chance that both schedules are selected, a 9% chance that only the inferior one is selected, and a 12 % chance that none of the schedules are selected. If both schedules are efficacious with a 20% response rate and a 30% progression rate after Week 8 (Day 57 + 3 days), then there is a 97% chance that at least one schedule is selected for further studies.

The effects of SAHA in combination with Erlotinib are assessed by tabulating events and summarizing duration, intensity, and the time to onset by dose level. Objective response rate and progression rate after Week 8 (Day 57 + 3 weeks) along with the respective 95% exact confidence intervals are provided. Time to response, response duration, and progression-free survival are listed and summarized (median, range and Kaplan-Meier estimated distribution are determined if appropriate). Summary statistics are provided for the pharmacokinetic parameters area under the curve, (AUC), maximum concentration of drug (C_{max}), time of occurrence for maximum drug concentration (T_{max}) of SAHA and Erlotinib during the first 2 treatment cycles of the RP2D after the MTDs have been established.

Treatment Plan and Treatment Duration: Baseline evaluations assess the patient's eligibility for the study. Patients are enrolled after meeting all eligibility criteria, and having completed all Screening procedures. Patients are expected to begin treatment as soon as possible after registration. Treatment with SAHA and Erlotinib is administered in capsule form on an outpatient basis. SAHA and Erlotinib are taken with food, i.e., within 30 minutes following a meal, if possible. Patient compliance with study medications is monitored by capsule count that occurs during each cycle. After receiving SAHA, patients are seen at regular intervals for assessment of efficacy and safety. Patients are treated until disease progression, intolerable toxicity, or the investigator determines that it is in the best interest of the patient to withdraw. Patients receive up to 6 months of SAHA and Erlotinib on this study. Patients who do not have disease progression, and who continue to meet the eligibility criteria after the first 8 cycles, are offered continued treatment with SAHA at the same dose and schedule in a continuation protocol.

Dose Modification and Treatment Delay: National Cancer Institute (NCI) Common Terminology for Adverse Events (CTCAE) version 3.0 is used to assess adverse events in this study. SAHA and Erlotinib may be held in the presence of Grade 3 or 4 non-drug-related toxicity if the physician feels it is unsafe to continue the administration. In the presence of Grade 3 or 4 drug-related toxicity, SAHA and Erlotinib are held until the toxicity resolves to Grade 1 or less (or baseline CTCAE grade, if higher than Grade 1). In the instance of Grade 3 anemia or thrombocytopenia, both drugs may be continued if in the opinion of the investigator, the toxicity can be managed. Patients are withdrawn from the study if they fail to recover to CTCAE Grade 0 or 1 (or within 1 grade of starting values for preexisting laboratory abnormalities) from a

treatment-related toxicity within 2 weeks (leading to treatment delay of > 2 weeks) of holding drug, unless the investigator feels that the patient should remain in the study because there is evidence that the patient is deriving benefit from continuing study treatment.

In the event of acute onset of new or progressive pulmonary symptoms such as dyspnea, cough or fever, treatment with Erlotinib and SAHA should be interrupted pending diagnostic evaluation. If interstitial lung disease (ILD) is diagnosed, the patient is discontinued from the study. These symptoms are not considered dose-limiting toxicities, if less than Grade 3, and not associated with ILD. In Part I or Part II of the study, in the event that an individual patient experiences a DLT other than rash, the SAHA and Erlotinib is held until the DLT resolves to Grade 1 intensity or less (or baseline CTCAE grade, if higher than Grade 1). Dose modification details are shown below in Table 2.

Table 2

SAHA/Erlotinib Intrapatient Dose Modification for Dose Limiting Toxicity other than Diarrhea or Rash, Part I and Part II

	Dose Modification #1	Dose Modification #2
SAHA Cohort A		
300 mg q.d. 3 out of 7 days 400 mg q.d. 3 out of 7 days 500 mg q.d. 3 out of 7 days	200 mg q.d. 3 out of 7 days 300 mg q.d. 3 out of 7 days 400 mg q.d. 3 out of 7 days	OFF STUDY 200 mg q.d. 3 out of 7 days 300 mg q.d. 3 out of 7 days
SAHA Cohort B		
200 mg b.i.d. 3 out of 7 days 300 mg b.i.d. 3 out of 7 days 300 mg b.i.d. 7 out of 14 days	300 mg q.d. continuous 200 mg b.i.d. 3 out of 7 days 300 mg b.i.d. 3 out of 7 days	300 mg q.d. 14 out of 28 days 300 mg q.d. continuous 200 mg b.i.d. 3 out of 7 days
Erlotinib Cohort A or B		
150 mg P.O. daily	100 mg P.O. daily	50 mg P.O. daily

Alternatively, intrapatient dose modifications for determining SAHA/Erlotinib dose-limiting toxicity are shown in Table 3.

Table 3

SAHA/Erlotinib Intrapatient Dose Modification for Dose Limiting Toxicity other than Rash Part I and Part II

	Dose Modification #1	Dose Modification #2
SAHA Cohort A		
400 mg q.d. 21 out of 28 days	400 mg q.d. 14 out of 28 days	300 mg q.d. 14 out of 28 days

400 mg q.d. continuous	400 mg q.d. 21 out of 28 days	400 mg q.d. 14 out of 28 days
500 mg q.d. continuous	400 mg q.d. continuous	400 mg q.d. 21 out of 28 days
SAHA Cohort B		
200 mg b.i.d. 3 out of 7 days	300 mg q.d. continuous	300 mg q.d. 14 out of 28 days
300 mg b.i.d. 3 out of 7 days	200 mg b.i.d. 3 out of 7 days	300 mg q.d. continuous
300 mg b.i.d. 7 out of 14 days	300 mg b.i.d. 3 out of 7 days	200 mg b.i.d. 3 out of 7 days
Erlotinib Cohort A or B		
150 mg P.O. daily	100 mg P.O. daily	50 mg P.O. daily

Table 4

Erlotinib Intrapatient Dose Modification for Dose Limiting toxicity of Diarrhea or Rash Part I and Part II

5

Erlotinib Cohort A or B	Dose Modification #1	Dose Modification #2
150 mg P.O. daily	100 mg P.O. daily	50 mg P.O. daily

Pharmacokinetic Samples: Once the RP2D has been determined, PK samples for SAHA and Erlotinib will be drawn in the first 8 patients treated on the Phase II component of the study for Cohort A and the first 8 patients treated on the Phase II component of the study for Cohort
10 B.. PK time points are drawn. Samples are drawn on Visit 2, (Day 1) and Visit 4, (Day 16) of Cycle 1 as well as on Visit 8 (Day 16) of Cycle 2. Summary statistics are provided (mean, standard deviation, median, and range) for PK parameters (AUC, C_{max}, T_{max}) of SAHA and Erlotinib when administered in combination at the recommended Phase II dose.

Efficacy Analyses and Overall Response Criteria: The primary efficacy measurement in
15 Part I determines the MTD of oral SAHA in combination with Erlotinib and establishes that this treatment is sufficiently safe and tolerable to permit further study. The primary efficacy measurement in Part II determines the objective response rate, and progression rate, and explores the time to response, response duration, and progression free survival, in patients treated with SAHA and Erlotinib in combination at the RP2D. Objective response rate and progression rate
20 at Week 8 along with the respective 95% exact confidence intervals are provided. Time to response, response duration, and progression free-survival are listed and summarized (median, range and Kaplan-Meier estimated distribution, if appropriate).

Objective response rate is defined as the proportion of patients with responses consisting of Complete Response (CR) or Partial Response (PR) based on CT scans using RECIST criteria (Therasse *et al.*, *J. Natl. Cancer Inst.* 2000 Feb 2;92(3):205-16). The minimum size of a target lesion is 10 mm for spiral CT and 20 mm for conventional CT. Confirmation of the initial response is by a second assessment performed within approximately 4 weeks. Sites take every effort to use the same imaging modality throughout the patient's study course. Target lesions are all measurable lesions up to a maximum of 5 lesions per organ and 10 lesions in total, representative of all involved organs. They are recorded and measured at baseline. All other lesions (or sites of disease) are identified as non target lesions and are also recorded at baseline. Measurements of non target lesions are not required, but the presence or absence of each should be noted throughout follow-up (Therasse *et al.*, *J. Natl. Cancer Inst.* 2000 Feb 2;92(3):205-16). For Radiographically (CT) defined lesions, CT Scans are performed at baseline, the end of the study, and Day 1 of Cycles 3, 5, and 7. At baseline, tumor lesions are categorized using Standard RECIST criteria (Therasse *et al.*, *J. Natl. Cancer Inst.* 2000 Feb 2;92(3):205-16).

While this invention has been particularly shown and described with references to particular embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the meaning of the invention described. The scope of the invention encompasses the claims that follow.

CLAIMS

What is claimed is:

1. A method of treating cancer in a subject in need thereof comprising administering to the subject a histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA), represented by the structure:

   or a pharmaceutically acceptable salt or hydrate thereof, and a tyrosine kinase inhibitor, Erlotinib, represented by the structure:

   or a pharmaceutically acceptable salt or hydrate thereof, wherein the histone deacetylase inhibitor and the tyrosine kinase inhibitor are administered in amounts effective for treating the cancer.

2. The method of claim 1, wherein the histone deacetylase inhibitor and the tyrosine kinase inhibitor are administered concurrently.

3. The method of claim 1, wherein the histone deacetylase inhibitor is administered prior to administering the tyrosine kinase inhibitor.

4. The method of claim 1, wherein the histone deacetylase inhibitor is administered after administering the tyrosine kinase inhibitor.

5. The method of claim 1, wherein the histone deacetylase inhibitor and the tyrosine kinase inhibitor are administered orally.

6. The method of any one of claims 1-5, wherein suberoylanilide hydroxamic acid (SAHA) and Erlotinib are administered.

7. The method of any one of claims 1-6, wherein the cancer is non-small cell lung cancer.

8. The method of any one of claims 1-7, wherein the histone deacetylase inhibitor is administered once daily at a dose of 300 mg, wherein the administration is continuous.

9. The method of any one of claims 1-7, wherein the histone deacetylase inhibitor is administered once daily at a dose of 200 mg for at least one period of 3 out of 7 days.

10. The method of any one of claims 1-7, wherein the histone deacetylase inhibitor is administered once daily at a dose of 300 mg for at least one period of 3 out of 7 days.

11. The method of any one of claims 1-7, wherein the histone deacetylase inhibitor is administered once daily at a dose of 400 mg for at least one period of 3 out of 7 days.

12. The method of any one of claims 1-7, wherein the histone deacetylase inhibitor is administered once daily at a dose of 500 mg for at least one period of 3 out of 7 days.

13. The method of any one of claims 1-7, wherein the histone deacetylase inhibitor is administered twice daily at 200 mg per dose for at least one period of 3 out of 7 days.

14. The method of any one of claims 1-7, wherein the histone deacetylase inhibitor is administered twice daily at 300 mg per dose for at least one period of 3 out of 7 days.

15. The method of any one of claims 9-14, wherein the histone deacetylase inhibitor is administered for at least one period of 3 out of 7 days for two weeks, followed by a two-week rest period.

16. The method of any one of claims 9-14, wherein the histone deacetylase inhibitor is administered for at least one period of 3 out of 7 days for three weeks, followed by a one-week rest period.

17. The method of any one of claims 9-14, wherein the histone deacetylase inhibitor is administered for at least one period of 3 out of 7 days for one week, followed by a one-week rest period.

18. The method of any one of claims 1-7, wherein the histone deacetylase inhibitor is administered twice daily at 300 mg per dose for at least one period of 7 out of 14 days.

19. The method of any one of claims 1-7, wherein the histone deacetylase inhibitor is administered once daily at 300 mg per dose for at least one period of 14 out of 28 days.

20. The method of any one of claims 1-19, wherein the tyrosine kinase inhibitor is administered once daily at a dose of 50 mg, wherein the administration is continuous.

21. The method of any one of claims 1-19, wherein the tyrosine kinase inhibitor is administered once daily at a dose of 100 mg, wherein the administration is continuous.

22. The method of any one of claims 1-19, wherein the tyrosine kinase inhibitor is administered once daily at a dose of 150 mg, wherein the administration is continuous.

23. The method of any one of claims 1-7, wherein the histone deacetylase inhibitor is administered at a total daily dose of up to 400 mg and the tyrosine kinase inhibitor is administered at a total daily dose of up to 150 mg.

24. The method of any one of claims 1-7, wherein the histone deacetylase inhibitor is administered at a total daily dose of up to 600 mg and the tyrosine kinase inhibitor is administered at a total daily dose of up to 150 mg.

25. An oral pharmaceutical composition comprising a histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA), represented by the structure:

or a pharmaceutically acceptable salt or hydrate thereof, and a tyrosine kinase inhibitor, Erlotinib, represented by the structure:

or a pharmaceutically acceptable salt or hydrate thereof, and optionally one or more pharmaceutically acceptable excipients.

26. The pharmaceutical composition of claim 25 that comprises about 100 mg of SAHA and about 50 mg of Erlotinib.

27. The pharmaceutical composition of claim 25, which comprises suberoylanilide hydroxamic acid (SAHA) and Erlotinib.

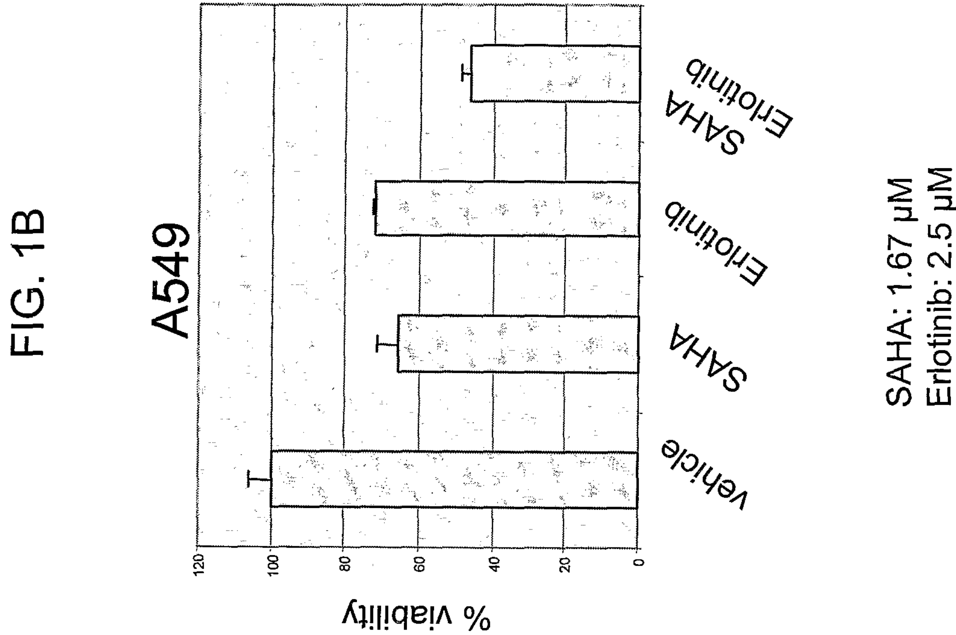
FIG. 1B
A549
% viability
120
100
80
60
40
20
0
vehicle
SAHA
Erlotinib
SAHA
Erlotinib
SAHA: 1.67 μM
Erlotinib: 2.5 μM

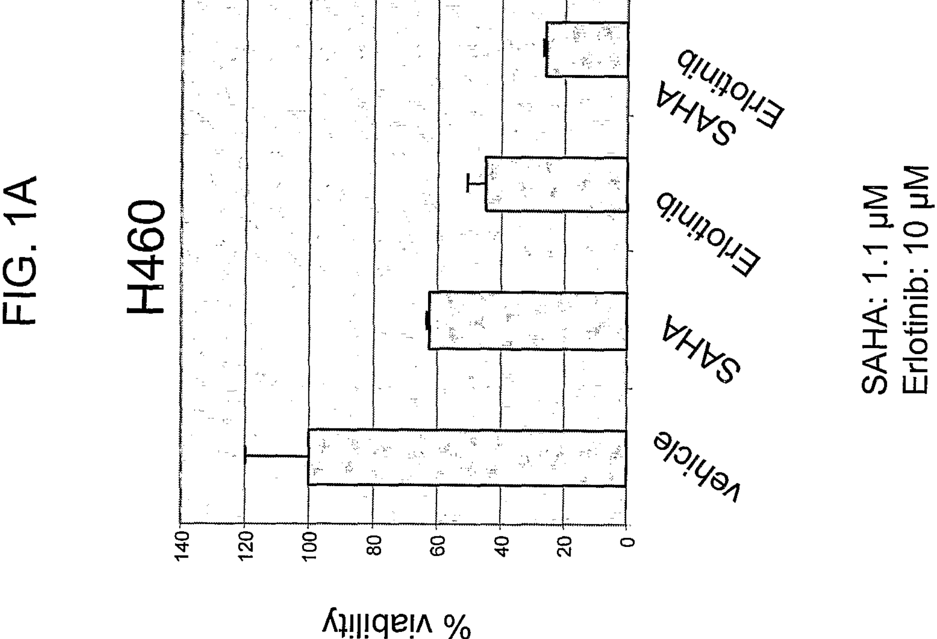
FIG. 1A
H460
% viability
140
120
100
80
60
40
20
0
vehicle
SAHA
Erlotinib
SAHA
Erlotinib
SAHA: 1.1 μM
Erlotinib: 10 μM