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[Continued on next page]

(54) Title: METHODS FOR THE TREATMENT OF CANCER

(57) Abstract: Described herein are methods for the treatment of cancer in a subject. In particular, methods are provided for the treatment of lung cancer with a combination of entinostat and an EGFR inhibitor, or the treatment of breast cancer with a combination of entinostat and an aromatase inhibitor. Furthermore, a food effect was evident for the oral administration of entinostat.

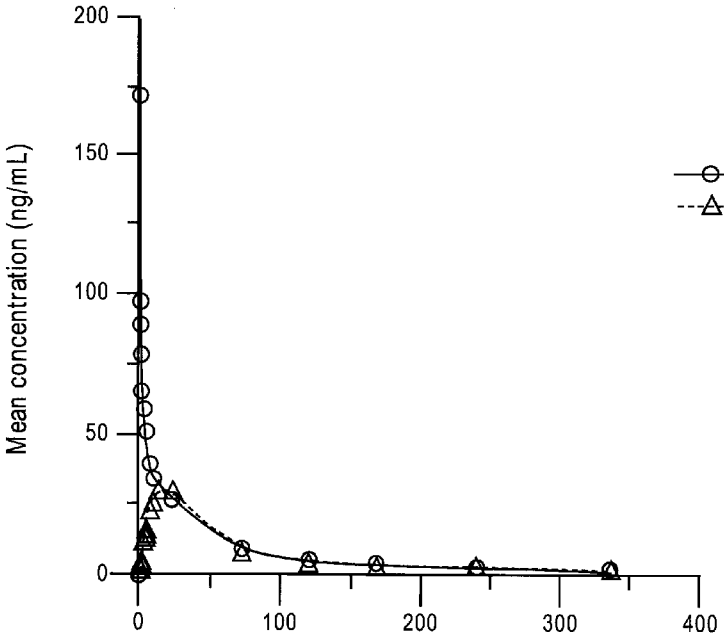

Figure 1(a)

WO 2014/179738 A1

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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METHODS FOR THE TREATMENT OF CANCER

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 61/819,505, filed May 3, 2013, which is incorporated herein by reference in its entirety.

FIELD

[0002] The present invention relates to methods for the treatment of cancer based on the co-administration HDAC inhibitors.

BACKGROUND

[0003] Cancer, tumors, tumor-related disorders, and neoplastic disease states are serious and often times life-threatening conditions. These diseases and disorders, which are characterized by rapidly-proliferating cell growth, continue to be the subject of research efforts directed toward the identification of therapeutic agents which are effective in the treatment thereof. Such agents prolong the survival of the patient, inhibit the rapidly-proliferating cell growth associated with the neoplasm, or effect a regression of the neoplasm.

[0004] Generally, surgery and radiation therapy are the first modalities considered for the treatment of cancer that is considered locally confined, and offer the best prognosis. Chemotherapy treatment of certain cancers typically results in disappointing survival rates but still offer a survival benefit. For example, in patients with lung cancer, epidermal growth factor receptor (EGFR) inhibitor chemotherapy regimens, such as the use of erlotinib and gefitinib are employed. If patients fail to respond to an EGFR inhibitor treatment, additional conventional treatment, as currently employed, offers limited benefit. In patients with breast cancer, aromatase inhibitor chemotherapy regimens, such as the use of letrozole, anastrozole or exemestane, are employed. If patients fail to respond to an aromatase inhibitor treatment, additional conventional treatment offers limited benefit.

[0005] While several EGFR inhibitors have been approved for the treatment of lung cancer, EGFR inhibitor therapy encounters limitations, such as side-effects resulting from its use. Of greater concern, is the growing view that, while utilization of EGFR inhibitors for the treatment of tumors may initially shrink the size of the tumor, the tumor may eventually enlarge in size, indicating, among other things, the development of resistance. Erlotinib, a widely used EGFR inhibitor, may be representative of the types of therapeutic agents being used for cancer treatment in that its use has an effect on cancer, but because of other factors, which are not entirely known, the tumor develops resistance and progresses.

[0006] Despite the approval of several aromatase inhibitors for the treatment of early and late stage breast cancer, as with most therapeutic agents, side-effects result from its use. For example, common side effects include hot flashes, vasodilation and nausea. Of greater concern, is the growing view that, while utilization of aromatase inhibitors for the treatment of tumors may initially shrink the size of the tumor, the tumor may eventually enlarge in size, indicating, among other things, the development of resistance. Letrozole, a widely used aromatase inhibitor, may be representative of the types of therapeutic agents being used for cancer treatment; in that its use has an effect on cancer, but because of other factors, which are not entirely known, the tumor develops resistance and progresses.

[0007] Histone deacetylase (HDAC) inhibitors are an emerging class of therapeutic agents that promote differentiation and apoptosis in hematologic and solid malignancies through chromatin remodeling and gene expression regulation. Several HDAC inhibitors have been identified including benzamides (entinostat), short-chain fatty acids (*i.e.*, Sodium phenylbutyrate); hydroxamic acids (*i.e.*, suberoylanilide hydroxamic acid and thrichostatin A); cyclic tetrapeptides containing a 2-amino-8-oxo-9, 10-epoxy-decanoyl moiety (*i.e.*, trapoxin A) and cyclic peptides without the 2-amino-8-oxo-9, 10-epoxy-decanoyl moiety (*i.e.*, FK228). Entinostat is a benzamide HDAC inhibitor undergoing clinical investigation in multiple types of solid tumors and hematologic cancers. Entinostat is rapidly absorbed and has a half-life of about 100 hours and, importantly, changes in histone acetylation persist for several weeks following the administration of entinostat.

[0008] What is needed, therefore, are compositions and/or methods of treatment for cancer which take advantage of the synergy found in a therapeutic combination that could increase the effectiveness of the agents and reduce and/or eliminate the side effects typically associated with conventional treatments.

SUMMARY OF THE INVENTION

[0009] One embodiment provides a method of treating cancer in a patient in need thereof, comprising oral administration of entinostat, wherein the Cmax of entinostat is increased when the entinostat is administered under fasting conditions, compared to when entinostat is administered under fed conditions.

[0010] One embodiment provides a method of treating cancer in a patient in need thereof, comprising oral administration of entinostat, wherein the Tmax of entinostat is increased when the entinostat is administered under fed conditions, compared to when entinostat is administered under fasting conditions.

[0011] One embodiment provides a method of treating breast cancer in a patient in need thereof, comprising oral administration of exemestane and entinostat, wherein the entinostat is administered to a fasting patient.

[0012] One embodiment provides a method of treating non-small cell lung cancer in a patient in need thereof, comprising oral administration of erlotinib and entinostat, wherein the entinostat is administered to a fasting patient.

[0013] One embodiment provides a method of treating cancer in a patient in need thereof, comprising oral administration of entinostat, wherein the administration of entinostat under fasting conditions results in an increase of the Cmax as compared to the administration of entinostat under fed conditions, and wherein the ratio of Cmax following administration under fasting conditions to Cmax following administration under fed conditions is at least about 2:1.

[0014] One embodiment provides a method of treating cancer in a patient in need thereof, comprising oral administration of entinostat, wherein the administration of entinostat under fed conditions results in an increase of the Tmax as compared to the administration of entinostat under fasting conditions, and wherein the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 2:1.

INCORPORATION BY REFERENCE

[0015] All publications, patents, and patent applications described in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. Patent applications PCT international patent application no. PCT/US2012/053551; US patent application no.14/342,354; and US patent application publication 2013/0150386 are herein incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

Figure 1 provides a pharmacokinetic analysis of the food effect study described in Example 1.

DETAILED DESCRIPTION

[0017] Provided herein are methods of treating breast cancer based on the administration of a histone deacetylase (HDAC) inhibitor and an aromatase inhibitor. The methods include administering the HDAC inhibitor without food. The methods of treatment may incorporate patient selections based on levels of protein lysine acetylation observed during treatment. The methods may further include treatments wherein the administration of the HDAC inhibitor and the aromatase inhibitor are supplemented with one or more therapeutic agents or therapies.

[0018] Provided herein are methods of treating lung cancer based on the administration of an HDAC inhibitor and an epidermal growth factor receptor (EGFR) inhibitor. The methods include administering the HDAC inhibitor without food. The methods may further include treatments wherein the administration of the HDAC inhibitor and the EGFR inhibitor are supplemented with one or more therapeutic agents or therapies.

[0019] One embodiment provides a method of treating cancer in a patient in need thereof, comprising oral administration of entinostat, wherein the administration of entinostat under fasting conditions results in an increase of the Cmax as compared to the administration of entinostat under fed conditions, and wherein the ratio of Cmax following administration under fasting conditions to Cmax following administration under fed conditions is at least about 2:1. Another embodiment provides the method of treating cancer wherein the ratio of Cmax following administration under fasting conditions to Cmax following administration under fed conditions is at least about 3:1. Another embodiment provides the method of treating cancer wherein the ratio of Cmax following administration under fasting conditions to Cmax following administration under fed conditions is at least about 4:1. Another embodiment provides the method of treating cancer wherein the ratio of Cmax following administration under fasting conditions to Cmax following administration under fed conditions is at least about 5:1. Another embodiment provides the method of treating cancer wherein the ratio of Cmax following administration under fasting conditions to Cmax following administration under fed conditions is at least about 6:1. Another embodiment provides the method of treating cancer wherein the ratio of Cmax following administration under fasting conditions to Cmax following administration under fed conditions is at least about 7:1. Another embodiment provides the method of treating cancer wherein the cancer is lung cancer. Another embodiment provides the method of treating cancer wherein the lung cancer is non-small cell lung cancer. Another embodiment provides the method of treating cancer wherein the cancer is breast cancer. Another embodiment provides the method of treating cancer further comprising oral administration of an EGFR inhibitor. Another embodiment provides the method of treating cancer wherein the EGFR inhibitor is erlotinib. Another embodiment provides the method of treating cancer wherein

the erlotinib is administered at a different time of day than entinostat. Another embodiment provides the method of treating cancer wherein the patient has not consumed food within 2 hours prior to administration or erlotinib. Another embodiment provides the method of treating cancer wherein the patient does not consume food within 1 hour after administration of erlotinib. Another embodiment provides the method of treating cancer wherein about 150 mg of erlotinib is administered. Another embodiment provides the method of treating cancer wherein the erlotinib is administered once daily. Another embodiment provides the method of treating cancer further comprising oral administration of an aromatase inhibitor. Another embodiment provides the method of treating cancer wherein the aromatase inhibitor is exemestane. Another embodiment provides the method of treating cancer wherein the exemestane is administered at a different time of day than entinostat. Another embodiment provides the method of treating cancer wherein exemestane is administered after a meal. Another embodiment provides the method of treating cancer wherein exemestane is administered with a meal. Another embodiment provides the method of treating cancer wherein about 25 mg of exemestane is administered. Another embodiment provides the method of treating cancer wherein the exemestane is administered once daily. Another embodiment provides the method of treating cancer wherein the patient is administered about 10 mg of entinostat. Another embodiment provides the method of treating cancer wherein the patient is administered about 5 mg of entinostat. Another embodiment provides the method of treating cancer wherein the patient is administered from about 1 mg to about 20 mg of entinostat. Another embodiment provides the method of treating cancer wherein the patient has not consumed food within 2 hours prior to administration of entinostat under fasting conditions.

Another embodiment provides the method of treating cancer wherein the patient has not consumed food within 1 hour prior to administration of entinostat under fasting conditions. Another embodiment provides the method of treating cancer wherein the patient does not consume food within 2 hours after administration of entinostat under fasting conditions. Another embodiment provides the method of treating cancer wherein the patient does not consume food within 30 minutes after administration of entinostat under fasting conditions. Another embodiment provides the method of treating cancer wherein the patient consumes a high fat meal under fed conditions.

[0020] One embodiment provides a method of treating cancer in a patient in need thereof, comprising oral administration of entinostat, wherein the administration of entinostat under fed conditions results in an increase of the Tmax as compared to the administration of entinostat under fasting conditions, and wherein the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 2:1. Another embodiment provides the method of treating cancer wherein the ratio of Tmax following administration under

fed conditions to Tmax following administration under fasting conditions is from about 2:1 to about 5:1. Another embodiment provides the method of treating cancer wherein the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is from about 5:1 to about 8:1. Another embodiment provides the method of treating cancer wherein the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is from about 8:1 to about 12:1. Another embodiment provides the method of treating cancer wherein the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is from about 12:1 to about 15:1. Another embodiment provides the method of treating cancer wherein the cancer is lung cancer. Another embodiment provides the method of treating cancer wherein the lung cancer is non-small cell lung cancer. Another embodiment provides the method of treating cancer wherein the cancer is breast cancer. Another embodiment provides the method of treating cancer further comprising oral administration of an EGFR inhibitor. Another embodiment provides the method of treating cancer wherein the EGFR inhibitor is erlotinib. Another embodiment provides the method of treating cancer wherein the erlotinib is administered at a different time of day than entinostat. Another embodiment provides the method of treating cancer wherein the patient has not consumed food within 2 hours prior to administration or erlotinib. Another embodiment provides the method of treating cancer wherein the patient does not consume food within 1 hour after administration of erlotinib. Another embodiment provides the method of treating cancer wherein about 150 mg of erlotinib is administered. Another embodiment provides the method of treating cancer wherein the erlotinib is administered once daily. Another embodiment provides the method of treating cancer further comprising oral administration of an aromatase inhibitor. Another embodiment provides the method of treating cancer wherein the aromatase inhibitor is exemestane. Another embodiment provides the method of treating cancer wherein the exemestane is administered at a different time of day than entinostat. Another embodiment provides the method of treating cancer wherein exemestane is administered after a meal. Another embodiment provides the method of treating cancer wherein exemestane is administered with a meal. Another embodiment provides the method of treating cancer wherein about 25 mg of exemestane is administered. Another embodiment provides the method of treating cancer wherein the exemestane is administered once daily. Another embodiment provides the method of treating cancer wherein the patient is administered about 10 mg of entinostat. Another embodiment provides the method of treating cancer wherein the patient is administered about 5 mg of entinostat. Another embodiment provides the method of treating cancer wherein the patient is administered from about 1 mg to about 20 mg of entinostat. Another embodiment provides the method of treating cancer wherein the patient has not consumed food

within 2 hours prior to administration of entinostat under fasting conditions. Another embodiment provides the method of treating cancer wherein the patient has not consumed food within 1 hour prior to administration of entinostat under fasting conditions. Another embodiment provides the method of treating cancer wherein the patient does not consume food within 2 hours after administration of entinostat under fasting conditions. Another embodiment provides the method of treating cancer wherein the patient does not consume food within 30 minutes after administration of entinostat under fasting conditions. Another embodiment provides the method of treating cancer wherein the patient consumes a high fat meal under fed conditions.

[0021] To facilitate understanding of the disclosure set forth herein, a number of terms are defined below.

[0022] As used herein, "abnormal cell growth," refers to cell growth that is independent of normal regulatory mechanisms (e.g., loss of contact inhibition), including the abnormal growth of normal cells and the growth of abnormal cells.

[0023] "Neoplasia" as described herein, is an abnormal, unregulated and disorganized proliferation of cells that is distinguished from normal cells by autonomous growth and somatic mutations. As neoplastic cells grow and divide they pass on their genetic mutations and proliferative characteristics to progeny cells. A neoplasm, or tumor, is an accumulation of neoplastic cells. In some embodiments, the neoplasm can be benign or malignant.

[0024] "Metastasis," as used herein, refers to the dissemination of tumor cells via lymphatics or blood vessels. Metastasis also refers to the migration of tumor cells by direct extension through serous cavities, or subarachnoid or other spaces. Through the process of metastasis, tumor cell migration to other areas of the body establishes neoplasms in areas away from the site of initial appearance.

[0025] As discussed herein, "angiogenesis" is prominent in tumor formation and metastasis. Angiogenic factors have been found associated with several solid tumors such as rhabdomyosarcomas, retinoblastoma, Ewing sarcoma, neuroblastoma, and osteosarcoma. A tumor cannot expand without a blood supply to provide nutrients and remove cellular wastes. Tumors in which angiogenesis is important include solid tumors such as renal cell carcinoma, hepatocellular carcinoma, and benign tumors such as acoustic neuroma, and neurofibroma. Angiogenesis has been associated with blood-born tumors such as leukemias. It is believed that angiogenesis plays a role in the abnormalities in the bone marrow that give rise to leukemia. Prevention of angiogenesis could halt the growth of cancerous tumors and the resultant damage to the subject due to the presence of the tumor.

[0026] The term "subject" refers to an animal, including, but not limited to, a primate (e.g., human), cow, sheep, goat, horse, dog, cat, rabbit, rat, or mouse. The terms "subject" and "patient" are used interchangeably herein in reference, for example, to a mammalian subject, such as a human subject.

[0027] The terms "treat," "treating," and "treatment" are meant to include alleviating or abrogating a disorder, disease, or condition; or one or more of the symptoms associated with the disorder, disease, or condition; or alleviating or eradicating the cause(s) of the disorder, disease, or condition itself.

[0028] The term "therapeutically effective amount" refers to the amount of a compound that, when administered, is sufficient to prevent development of, or alleviate to some extent, one or more of the symptoms of the disorder, disease, or condition being treated. The term "therapeutically effective amount" also refers to the amount of a compound that is sufficient to elicit the biological or medical response of a cell, tissue, system, animal, or human that is being sought by a researcher, veterinarian, medical doctor, or clinician.

[0029] The term "pharmaceutically acceptable carrier," "pharmaceutically acceptable excipient," "physiologically acceptable carrier," or "physiologically acceptable excipient" refers to a pharmaceutically-acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, excipient, solvent, or encapsulating material. Each component must be "pharmaceutically acceptable" in the sense of being compatible with the other ingredients of a pharmaceutical formulation. It must also be suitable for use in contact with the tissue or organ of humans and animals without excessive toxicity, irritation, allergic response, immunogenicity, or other problems or complications, commensurate with a reasonable benefit/risk ratio. S*ee, Remington: The Science and Practice of Pharmacy*, 21st Edition; Lippincott Williams & Wilkins: Philadelphia, PA, 2005; *Handbook of Pharmaceutical Excipients*, 5th Edition; Rowe et al., Eds., The Pharmaceutical Press and the American Pharmaceutical Association: 2005; and *Handbook of Pharmaceutical Additives*, 3rd Edition; Ash and Ash Eds., Gower Publishing Company: 2007; *Pharmaceutical Preformulation and Formulation*, Gibson Ed., CRC Press LLC: Boca Raton, FL, 2004).

[0030] The term "pharmaceutical composition" refers to a mixture of a compound disclosed herein with other chemical components, such as diluents or carriers. The pharmaceutical composition facilitates administration of the compound to an organism. Multiple techniques of administering a compound exist in the art including, but not limited to, oral, injection, aerosol, parenteral, and topical administration. Pharmaceutical compositions can also be obtained by reacting compounds with inorganic or organic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric

acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

[0031] The terms "fasting", "fasted" or "without food" are defined to mean, in general, the condition of not having consumed food during the period between from at least about 30 minutes prior to the administration of a therapeutic agent described herein to at least about 30 minutes after the administration of a therapeutic agent described herein. In some instances, food is not consumed from at least about 2 hours prior to the administration of a therapeutic agent described herein to at least about 1 hour after the administration of a therapeutic agent described herein. In some instances, food is not consumed from at least about 1 hours prior to the administration of a therapeutic agent described herein to at least about 1 hour after the administration of a therapeutic agent described herein. In some instances, food is not consumed from at least about 1 hours prior to the administration of a therapeutic agent described herein to at least about 2 hour after the administration of a therapeutic agent described herein.

[0032] The term "fed condition" refers to the condition of having eaten a meal. In some instances the food is a high fat or a high calorie meal. A high calorie meal can include, but is not limited to, a meal comprising 500 calories or more, from about 300 to about 800 calories, from about 500 calories to about 1,000 calories, and from about 800 calories to about 1,500 calories. In some instances, a high fat meal includes, but is not limited to, a calorie from fat percentage of a daily caloric intake from about 20% to about 50%, from about 30 to about 60%, and from about 40 to about 70%. In some embodiments, the meal is not high fat. In some embodiments, the meal is not high calorie.

[0033] The term "bioavailability" generally means the rate and extent to which an active ingredient is absorbed from a therapeutic agent and becomes available at the site of action. For oral dosage forms, bioavailability relates to the processes by which the active ingredient is released from the oral dosage form and moves to the site of action. Quantitatively, the term "oral bioavailability" or "%F" is defined as AUC_{oral}/AUC_{iv}, wherein AUC_{oral} is the AUC determined after oral administration and AUC_{iv} is the AUC determined after iv administration.

[0034] "AUC" refers to the area under the drug-concentration curve. "AUC^{0-t}" refers to the area under the drug-concentration curve from zero to time t. "AUClast" refers to the area under the drug-concentration curve from zero to last data point of drug-concentration curve. "$AUC^{0-\infty}$" or "AUCinf" refers to the area under the drug-concentration curve from zero to infinite time.

[0035] "$t_{1/2}$" refers to the elimination half-life of the indicated species. "t_{max}" refers to the time of the maximum concentration of the indicated species. "C_{max}" refers to the maximum concentration of the indicated species.

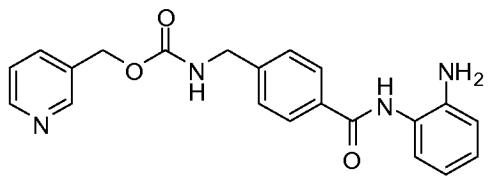
Treatment of Breast Cancer

Histone Deacetylase

[0036] The HDACs are a family including at least eighteen enzymes, grouped in three classes (Class I, II and III). Class I HDACs include, but are not limited to, HADCs 1, 2, 3, and 8. Class I HDACs can be found in the nucleus and are believed to be involved with transcriptional control repressors. Class II HDACs include, but are not limited to, HDACS 4, 5, 6, 7, and 9 and can be found in both the cytoplasm as well as the nucleus. Class III HDACs are believed to be NAD dependent proteins and include, but are not limited to, members of the Sirtuin family of proteins. Non-limiting examples of sirtuin proteins include SIRT1-7. As used herein, the term "selective HDAC" refers to an HDAC inhibitor that does not interact with all three HDAC classes.

HDAC Inhibitors

[0037] HDAC inhibitors can be classified broadly into pan HDAC inhibitors and selective HDAC inhibitors. Although there is a large structural diversity of known HDAC inhibitors, they share common features: a part that interacts with the enzyme active site and a side-chain that sits inside the channel leading to the active site. This can be seen with the hydroxamates such as SAHA, where the hydroxamate group is believed to interact with the active site. In the case of the depsipeptides, it is believed that an intracellular reduction of the disulphide bond creates a free thiol group (which interacts with the active site) attached to a 4-carbon alkenyl chain. A difference between the HDAC inhibitors is in the way that they interact with the rim of the HDAC channel, which is at the opposite end of the channel to the active site. It is this interaction, between the HDAC inhibitor and the rim of the channel, which is believed to account, at least in part, for some observed differences in HDAC selectivity between pan-HDAC inhibitors, such as SAHA and selective HDAC inhibitors such as the depsipeptides. A particularly preferred HDAC inhibitor is entinostat. Entinostat has the chemical name N-(2-aminophenyl)-4-[N-(pyridine-3-yl)methoxycarbonylamino-methyl]-benzamide and the chemical structure shown below.



Chemical structure of entinostat

Aromatase

[0038] Estrogen is one of the female sex hormones and has many functions in the body. It has been found that about 80% of breast cancer tumors overexpress the estrogen receptor and respond

positively to the presence of estrogen. In postmenopausal women, ovarian estrogen production is reduced and plasma estrogen levels are generally lower than in premenopausal women.

[0039] A residual source of estrogen in post-menopausal women is the synthesis of estrogens from androgens, which is catalyzed by aromatase. Inhibition of aromatase activity should lead to a reduction in the levels of estrogen and therefore a reduction in the growth of breast cancer tumors which respond positively to the presence of estrogen.

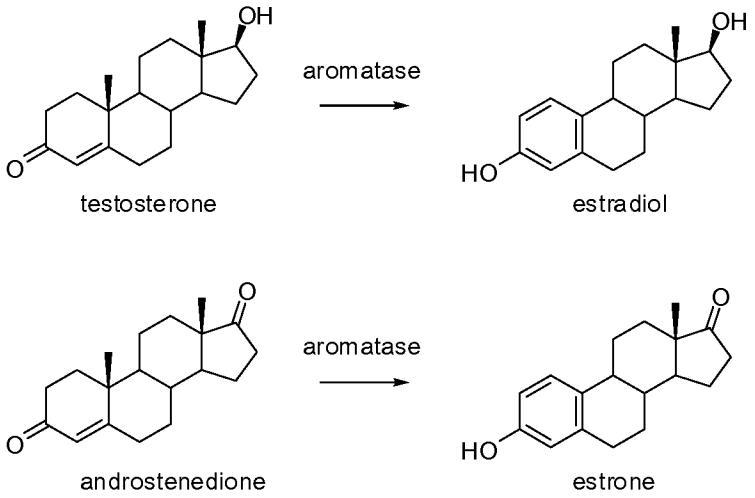
Aromatase is an enzyme of the cytochrome P450 family and a product of the CYP19 gene. The chemical function of aromatase is to convert testosterone to estradiol and androstenedione to estrone.

Aromatase Inhibitors

[0040] Aromatase inhibitors decrease the body's estrogen by blocking the enzyme aromatase from turning androgen into estrogen. For the treatment of early stage breast cancer, certain aromatase inhibitors may be used as adjuvant therapy instead of tamoxifen or after 2 or more years of tamoxifen. For the treatment of metastatic breast cancer, aromatase inhibitors are being tested in clinical trials to compare them to hormone therapy with tamoxifen.

[0041] As described herein, an "aromatase inhibitor" is a molecule which inhibits the activity of the aromatase enzyme. Compounds which are inhibitors of aromatase can be readily identified by one skilled in the art using methods such as, for example, standard pharmacological test procedures which measure the inhibition of the conversion of 1,2-^{3}H-androstenedione to estrone.

[0042] In brief, a microsomal fraction is prepared from human placenta by the method as described by Thompson and Siiteri (J. Biol. Chem., Vol. 249, p. 5364 (1974)). The microsomal preparation so obtained is lyophilized and stored at -40 °C. The human placental microsomes are added to 1,2-^{3}H-androstenedione and incubated for 20 minutes at 37 °C. The amount of aromatization of the labelled substrate is detected by the loss of $^{3}H_2O$ into the incubation medium. The substrate is removed by chloroform extraction, followed by adsorption to charcoal in suspension. The charcoal is removed by centrifugation and the steroid-free medium is counted in a liquid scintillation counter. Compositions are tested for aromatase inhibitory activity by adding them to the incubation medium prior to the addition of the microsomes. The relative cpm obtained with and without the composition is used to calculate the percent inhibition of the aromatization of androstenedione to estrone. IC_{50} values can be determined graphically as the concentration of test composition at which the aromatization of androstenedione to estrone is reduced to 50% of control value.

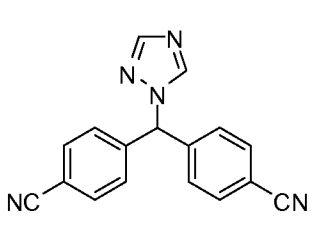


[0043] Subcutaneous fat is a major site of aromatase activity and it has been suggested that plasma estrogen levels correlate with body-mass index (Longcope et al , Metabolism 1986, 35, 235-7). It has been suggested that at menopause, plasma estrogen levels fall from about 110 pg/mL to a much lower level of about 7 pg/mL. However, in post-menopausal women, the intra-tumoral concentration of estradiol has been found to be about 10 times higher than in the plasma, probably due to aromatase activity within the tumor.

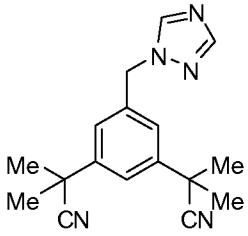
[0044] Inhibition of aromatase as a treatment option for breast cancer has been studied with some success. Currently three aromatase inhibitors are approved for marketing in the US for the treatment of breast cancer, at various stages, in post-menopausal women. Letrozole (Femara®) is indicated for several treatment options including, extended adjuvant treatment of early breast cancer in postmenopausal women with 5 years prior tamoxifen treatment, treatment of post menopausal women with hormone receptor positive (or unknown) locally advanced or metastatic breast cancer and advanced breast cancer treatment in postmenopausal women with disease progression following antiestrogen therapy.

[0045] Anastrozole (Arimidex®) is indicated for several treatment options including, adjuvant treatment of postmenopausal women with hormone receptor-(+) early breast cancer, first-line treatment of post menopausal women with hormone receptor-(+) (or unknown) locally advanced or metastatic breast cancer and advanced breast cancer in postmenopausal women with disease progression following tamoxifen therapy.

[0046] Exemestane (Aromasin®) is indicated for several treatment options including, adjuvant treatment of postmenopausal women with estrogen-receptor-(+) early breast cancer who have received 2-3 years of tamoxifen treatment and advanced breast cancer in postmenopausal women with disease progression following tamoxifen therapy.



Letrozole



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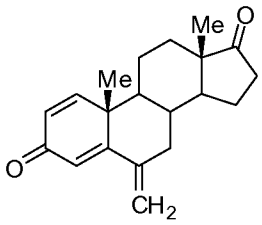

Exemestane

These drugs are grouped into two classes: (Type 1) exemestane is based on a steroid chemical structure and (type 2) letrozole and anastrozole are based on a non-steroidal chemical structure. Clinical trials have shown letrozole to be superior to tamoxifen in the treatment of advanced ER(+) disease. In early disease, adjuvant therapy with anastrozole appears to be superior to therapy with tamoxifen in reducing risk of relapse. Recent clinical trial results have led to aromatase inhibitors replacing tamoxifen as the standard of care for breast cancer treatment.

Breast Cancer

[0047] Today, among women in the United States, breast cancer remains the most frequent diagnosed cancer. One in 8 women in the United States is at risk of developing breast cancer. Age, family history, diet, and genetic factors have been identified as risk factors for breast cancer. Breast cancer is the second leading cause of death among women.

HER2/*neu* positive Breast Cancer

[0048] Cancers associated with overexpression of HER2/neu include breast, ovarian, endometrial, prostate, gastric, salivary gland, pancreatic, colorectal, oral and non-small cell lung cancers. Breast cancer has been a focus of anti-HER2/neu treatments.

[0049] Approximately 25-30 percent of breast cancers have an amplification of the HER2/neu gene or overexpression of its protein product. Overexpression of this receptor in breast cancer is associated with increased disease recurrence and worse prognosis.

Hormone Positive Cancer

[0050] Many breast cancers require the hormone estrogen to grow. In women who have had their menopause, the main source of estrogen is through the conversion of androgens into estrogens. As discussed above, this process is carried out by the aromatase enzyme.

Triple Negative Breast Cancer

[0051] In the treatment of triple negative breast cancer wherein the cancer is estrogen receptor-negative, progesterone receptor-negative and HER2-negative, compositions and therapies described herein may be combined with other therapeutic agents. Such agents include, by way of example only, cetuximab, paclitaxel, docetaxel, taxane formulations, for example, Abraxane® (ABI-007), Paclitaxel-Cremophor EL, Paclitaxel poliglumex, and Paclitaxel injectable emulsion (PIE). These

combinations may be advantageous when the cancer association with HER2 overexpression is present but undetected due to technical limitations in tests employed in quantifying HER 2 expression.

[0052] Hormonal therapies are the mainstay of treatment of estrogen receptor positive (ER+) breast cancer (BC). Due to both the clinical activity and the overall favorable side effect profile and tolerance of hormonal agents, the standard of care typically involves sequencing of hormonal agents until either the development of resistance and/or visceral crises necessitate switching to chemotherapy. In post-menopausal women the aromatase inhibitors (AI) are a preferred class of anti-estrogen therapy that functions by blocking endogenous estrogen synthesis. Exemestane is a steroidal AI which irreversibly binds and inactivates the aromatase enzyme with demonstrated efficacy in the metastatic setting after progression on a non-steroidal AI, NSAI; i.e. letrozole or anastrozole (Chia S, Gradishar W, Mauriac L, et al: Double-blind, randomized placebo controlled trial of fulvestrant compared with exemestane after prior nonsteroidal aromatase inhibitor therapy in postmenopausal women with hormone receptor–positive, advanced breast cancer: results from EFECT. J Clin Oncol 26:1664-1670, 2008).

[0053] The development of resistance to hormone therapies in advanced BC represents a significant challenge. Putative mechanisms of resistance include estrogen-independent growth, hypersensitivity to low estrogen concentrations, cyclin D1 over-expression, constitutive nuclear factor kappa B (NFκB) activation, up-regulation of growth factor signaling pathways and down-regulation of estrogen receptor alpha (ERα) expression. These pathways and mechanisms provide potential targets for therapeutic interventions. Entinostat is a novel, oral inhibitor of histone deacetylases (HDAC), with high specificity towards class 1 HDACs and a unique pharmacological profile allowing for weekly dosing. HDAC inhibition leads to elevated protein lysine acetylation in tumor and peripheral blood cells serving as a surrogate potential pharmacodynamic marker of activity. Entinostat's class 1 specificity distinguishes it from the United States (US) Food and Drug Administration (FDA)-approved HDAC inhibitors (HDACi) vorinstat (Zolinza®) and romidepsin (Istodax®). Preclinically, entinostat has demonstrated inhibition of ERα positive tumor growth and restoration of hormone sensitivity as a result of down-regulation of estrogen-independent growth factor signaling pathways, normalization of ERα levels and increases in aromatase enzyme levels. (Sabnis GJ, Goloubeva O, Chumsri S, et al: Functional activation of the estrogen receptor-α and aromatase by the HDAC inhibitor entinostat sensitizes ER-negative tumors to letrozole. Cancer Res 71:1893-903, 2011; Sabnis GJ, Kazi A, Goloubeva O, Brodie AMH. HDAC Inhibitor Entinostat Restores Responsiveness of Letrozole Resistant MCF-7Ca Xenografts to AIs through Modulation of Her-2. Presented at the 33rd Annual San Antonio Breast Cancer Symposium, San Antonio, TX,

December 8-12, 2010). The particular clinical trial results described herein demonstrate that combining entinostat with exemestane in ER+ breast cancers inhibits mechanisms of hormone therapy resistance thereby sensitizing cells to anti-estrogen therapy with exemestane.

Additional Therapy

[0054] Available additional treatments for breast cancer that may be advantageously employed in combination with the therapies disclosed herein include, without limitation, radiation therapy, chemotherapy, antibody therapy, and tyrosine kinase inhibitors as adjuvant therapy.

[0055] Radiation therapy is a cancer treatment that uses high-energy x-rays or other types of radiation to kill cancer cells or keep them from growing. Chemotherapy is a cancer treatment that uses drugs to stop the growth of cancer cells, either by killing the cells or by stopping them from dividing. When chemotherapy is taken by mouth or injected into a vein or muscle, the drugs enter the bloodstream and can reach cancer cells throughout the body (systemic chemotherapy). When chemotherapy is placed directly into the spinal column, an organ, or a body cavity such as the abdomen, the drugs mainly affect cancer cells in those areas (regional chemotherapy). The way the chemotherapy is given depends on the type and stage of the cancer being treated.

[0056] Different chemotherapeutic agents are known in the art for treating breast cancer. Cytoxic agents used for treating breast cancer include doxorubicin, cyclophosphamide, methotrexate, 5-fluorouracil, mitomycin C, mitoxantrone, paclitaxel, taxane formulations such as by way of example only, Abraxane® (ABI-007), Paclitaxel-Cremophor EL, Paclitaxel poliglumex, and Paclitaxel injectable emulsion (PIE), gemcitabine, docetaxel, capecitabine and epirubicin.

[0057] Other chemotherapy against breast cancer includes treatment with one or more of bendamustine, carboplatin (for example, Paraplatin®), carmustine (for example, BCNU®), chlorambucil (for example, Leukeran®), cisplatin (for example, Platinol®), cyclophosphamide injection (for example, Cytoxan®), oral cyclophosphamide (for example, Cytoxan®), dacarbazine (for example, DTIC®), ifosfamide (for example, ifex®), lomustine (for example, CCNU®), mechlorethamine (for example, nitrogen mustard, Mustargen®), melphalan (for example, Alkeran®), procarbazine (for example, Matulane®), bleomycin (for example, Blenoxane®), doxorubicin (for example, Adriamycin®, Rubex®), epirubicin, Idarubicin (for example, Idamycin®), mitoxantrone (for example, Novantrone®), gemcitabine (for example, Gemzar®), oral mercaptopurine (for example, Purinethol®). methotrexate, pentostatin IV (for example, Nipent®), oral thioguanine (for example, Lanvis®), oral etoposide (for example, VP-16, VePesid®, Etopophos) - etoposide IV (for example, VP-16, VePesid®, Etopophos), vinblastine (for example, Velban®), vincristine (for example, Oncovin®), vinorelbine (for example, Navelbine®),

dexamethasone (for example, Decadron®), methylprednisolone (for example, Medrol®), and prednisone (for example, Deltasone®).

[0058] Monoclonal antibody therapy is a cancer treatment that uses antibodies made in the laboratory, from a single type of immune system cell. These antibodies can identify substances on cancer cells or normal substances that may help cancer cells grow. The antibodies attach to the substances and kill the cancer cells, block their growth, or keep them from spreading. Monoclonal antibodies are given by infusion. They may be used alone or to carry drugs, toxins, or radioactive material directly to cancer cells. Monoclonal antibodies are also used in combination with chemotherapy as adjuvant therapy.

[0059] Trastuzumab (Herceptin®) is a monoclonal antibody that blocks the effects of the growth factor protein HER2, which transmits growth signals to breast cancer cells.

[0060] Trastuzumab leads to clinical responses as a single agent and improves survival when added to chemotherapy for advanced HER2-positive breast cancer. However, some patients do not respond to trastuzumab, and most eventually develop clinical resistance. Mechanisms of intrinsic and acquired trastuzumab resistance are poorly understood. One study which utilized a cell line-based approach to delineate genetic and protein alterations associated with resistance has been reported (D. Tripathy et al Journal of Clinical Oncology, 2005 Vol 23, No 16S, 3121). These researchers studied two HER2-positive breast cancer cell lines (BT474 and SKBR3) that were serially passaged in the presence of trastuzumab until *in vitro* resistance was documented. Resistant cell lines emerged after 12 months and exhibited a 3-fold more rapid growth rate in the absence of trastuzumab. Following trastuzumab exposure, G_0/G_1 arrest was observed in sensitive compared to resistant cells (84 vs. 68%), with fewer cells in S-phase (3 vs. 14%). Resistant cell lines exhibited fewer changes in gene expression with trastuzumab as well as upregulation of the chemokine receptor CXCR4 and mitotic checkpoint regulators, and downregulation of PTEN compared to sensitive cells.

[0061] Additional, illustrative, treatments that may be advantageously combined with the compositions and therapies disclosed herein may include, without limitation, administration of agents including, but not limited to lapatinib, alone or in combination with capecitabine, docetaxel, epirubicin, epothilone A, B or D, goserelin acetate, paclitaxel, pamidronate, bevacizumab, or trastuzumab.

[0062] In some embodiments, the additional therapy comprises chemotherapy comprising administering to the subject one or more of doxorubicin, cyclophosphamide, paclitaxel, lapatinib, capecitabine, trastuzumab, bevacizumab, gemcitabine, eribulin, or nab-paclitaxel.

Methods for the Treatment of Breast Cancer

[0063] One embodiment provides a method of treating breast cancer in a patient comprising (i) measuring the level of protein lysine acetylation prior to administration of entinostat-aromatase inhibitor combination therapy, (ii) administering entinostat-aromatase inhibitor combination therapy, (iii) measuring the level of protein lysine acetylation after administration of entinostat-aromatase inhibitor combination therapy, (iv) comparing the level of protein lysine acetylation after administration of entinostat-aromatase inhibitor combination therapy with the level of protein lysine acetylation prior to administration of entinostat-aromatase inhibitor combination therapy, and (v) continuing treatment with entinostat-aromatase inhibitor combination therapy if the level of protein lysine acetylation after administration of entinostat-aromatase inhibitor combination therapy is greater than the level of protein lysine acetylation prior to administration of entinostat-aromatase inhibitor combination therapy. In some instances, entinostat is administered to a fasting patient.

[0064] One embodiment provides a method of treating breast cancer in a patient comprising (i) administring entinostat-aromatase inhibitor combination therapy, and (ii) determining the change in protein lysine acetylation levels during the course of said therapy compared to pre-therapy protein lysine acetylation levels. In some instances, entinostat is administered to a fasting patient.

[0065] One embodiment provides a method of treating breast cancer in a patient comprising (i) determining the level prior to administration of protein lysine acetylation, (ii) administring entinostat-aromatase inhibitor combination therapy, and (iii) determining the level of protein lysine acetylation during the course of therapy. In some instances, entinostat is administered to a fasting patient.

[0066] It is desirable to increase the oral bioavailability of therpeutic agents, such as entinostat, to increase the extent of the therapeutic effect on the patient. In general, food has a variable effect on the bioavailability of a therapeutic agent. Interactions between a therapeutic agent and food may result in reduced, delayed or increased systemic drug availability. Food may interact with a therapeutic agent at the following phases: (i) before and during gastrointestinal absorption; (ii) during distribution; (iii) during metabolism; and (iv) during elimination. In one embodiment, entinostat bioavailability decreases when administered with food.

[0067] Food can affect peak exposure (Cmax) and time to peak exposure (Tmax) by delaying gastric emptying and prolonging intestinal transit time. In some instances, food affects the total exposure, or area under the concentration-time curve (AUC). In some embodiments, the Cmax is higher when entinostat is administered without food as compared to the Cmax when entinostat is administered with food. In some embodiments, the ratio of Cmax following administration of

entinostat under fasting conditions to Cmax following administration of entinostat under fed conditions is at least about 2:1. In one embodiment, the ratio of Cmax following administration of entinostat under fasting conditions to Cmax following administration of entinostat under fed conditions is at least about 3:1. In one embodiment, the ratio of Cmax following administration of entinostat under fasting conditions to Cmax following administration of entinostat under fed conditions is at least about 4:1. In one embodiment, the ratio of Cmax following administration of entinostat under fasting conditions to Cmax following administration of entinostat under fed conditions is at least about 5:1. In one embodiment, the ratio of Cmax following administration of entinostat under fasting conditions to Cmax following administration of entinostat under fed conditions is at least about 6:1. In one embodiment, the ratio of Cmax following administration of entinostat under fasting conditions to Cmax following administration of entinostat under fed conditions is at least about 7:1.

[0068] In some embodiments, the Tmax is lower when entinostat is administered without food as compared to the Tmax when entinostat is administered with food. In some embodiments, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 2:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 3:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 4:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 5:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 6:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 7:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 8:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 9:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 10:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 11:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 12:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under

fasting conditions is at least about 13:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 14:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 15:1.

[0069] In some embodiments, exemestane is administered a different time of day than entinostat. In one embodiment, exemstane is administerd after a meal. In one embodiemt, exemstane is administerd with a meal.

[0070] Another embodiment provides the method wherein determining the change in protein lysine acetylation level during the course of said therapy occurs after about 2 days of therapy, about 5 days of therapy, about 7 days of therapy, about 15 days of therapy, or about 21 days of therapy.

[0071] Another embodiment provides the method wherein the protein lysine acetylation levels are obtained from a tissue sample selected from B-cells, T-cells, or monocytes.

[0072] Another embodiment provides the method wherein the aromatase inhibitor is exemestane. Another embodiment provides the method wherein the aromatase inhibitor is anasrozole. Another embodiment provides the method wherein the aromatase inhibitor is letrozole. Another embodiment provides the method wherein the aromatase inhibitor is administered daily. Another embodiment provides the method wherein the aromatase inhibitor is exemestane and is administered daily. Another embodiment provides the method wherein etinostat is administered every 7 days of a 28-day cycle. Another embodiment provides the method wherein etinostat is administered every 14 days of a 28-day cycle. Another embodiment provides the method wherein the entinostat-aromatase inhibitor combination therapy comprises oral administration of entinostat every 7 days of a 28-day cycle, and oral administration of exemestane every day. Another embodiment provides the method wherein the entinostat-aromatase inhibitor combination therapy comprises oral administration of entinostat every 14 days of a 28-day cycle, and oral administration of exemestane every day. Another embodiment provides the method wherein etinostat is administered to a fasting patient every 7 days of a 28-day cycle. Another embodiment provides the method wherein etinostat is administered to a fasting patient every 14 days of a 28-day cycle. Another embodiment provides the method wherein the entinostat-aromatase inhibitor combination therapy comprises oral administration of entinostat to a fasting patient every 7 days of a 28-day cycle, and oral administration of exemestane every day. Another embodiment provides the method wherein the entinostat-aromatase inhibitor combination therapy comprises oral administration of entinostat to a fasting patient every 14 days of a 28-day cycle, and oral administration of exemestane every day.

[0073] Another embodiment provides the method wherein the step of determining the protein lysine acetylation level during the course of therapy is performed more than once. Another embodiment provides the method wherein the step of determining the protein lysine acetylation level during the course of therapy is performed once.

[0074] Another embodiment provides the method further comprising selecting the patient for further treatment if the level of protein lysine acetylation level increases during the course of therapy.

[0075] Another embodiment provides the method further comprising selecting the patient for further treatment if the level of protein lysine acetylation level increases during the first week of the course of therapy. Another embodiment provides the method further comprising selecting the patient for further treatment if the level of protein lysine acetylation level increases during the first and second week of the course of therapy.

[0076] One embodiment provides a method of selecting a patient for further entinostat-aromatase inhibitor combination therapy comprising comparing the protein lysine acetylation level in a tissue sample obtained after initiating therapy to the protein lysine acetylation levels determined prior to initiating therapy.

[0077] One embodiment provides a method of selecting a patient for further entinostat-aromatase inhibitor combination therapy comprising comparing the protein lysine acetylation level in a tissue sample obtained after initiating therapy to the protein lysine acetylation levels determined prior to initiating therapy, wherein an increase in protein lysine acetylation level after initiating therapy indicates the patient will benefit from further therapy.

[0078] Another embodiment provides the method wherein the protein lysine acetylation level in a tissue sample obtained after initiating therapy is determined more than once. Another embodiment provides the method wherein increase in protein lysine acetylation level after initiating therapy occurs over a time period of one week. Another embodiment provides the method wherein the protein lysine acetylation level after initiating therapy is determined on days 2, 8 and 15.

[0079] Another embodiment provides the method wherein the increase is from about 10 % to about 500 %. Another embodiment provides the method wherein the increase is from about 10 % to about 400 %. Another embodiment provides the method wherein the increase is from about 10 % to about 300 %. Another embodiment provides the method wherein the increase is from about 10 % to about 200 %. Another embodiment provides the method wherein the increase is from about 10 % to about 100 %. Another embodiment provides the method wherein the increase is about 10%, about 20%, about 30%, about 40%, about 50% or about 60%. Another embodiment provides the

method wherein the increase is about 25%, about 50%, about 75%, about 100%, about 125% or about 150%.

[0080] Another embodiment provides the method wherein the tissue sample is selected from B-cells, T-cells, or monocytes.

[0081] Another embodiment provides the method wherein the tissue sample obtained after initiating therapy is obtained at least 2 days after initiating therapy. Another embodiment provides the method wherein the tissue sample obtained after initiating therapy is obtained between day 2 and day 28 after initiating therapy. Another embodiment provides the method wherein the tissue sample obtained after initiating therapy is obtained on day 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 after initiating therapy.

[0082] One embodiment provides a method of selecting a patient for further entinostat-aromatase inhibitor combination therapy comprising comparing the percent change in protein lysine acetylation levels in a tissue sample obtained after initiating therapy to the protein lysine acetylation levels determined prior to initiating therapy, wherein a percent decrease in protein lysine acetylation levels after initiating therapy of about 5 percent to about 50 percent indicates the patient will not benefit from further therapy.

[0083] One embodiment provides a method of treating breast cancer which displays resistance to prior aromatase inhibitor therapy, the method comprising administering to a patient a combination comprising entinostat and an aromatase inhibitor, wherein the patient did not demonstrate a complete response, a partial response or stable disease for greater than six months during prior treatment with an aromatase inhibitor. In some instances, entinostat is administered to a fasting patient.

[0084] Another embodiment provides the method wherein the patient relapsed during treatment on or within 6 months of completion of prior non-steroidal aromatase inhibitor given as adjuvant therapy.

[0085] Another embodiment provides the method wherein the patient demonstrated progressive disease after at least 3 months treatment on prior non-steroidal aromatase inhibitor.

[0086] Another embodiment provides the method wherein the breast cancer is ER-positive.

[0087] Another embodiment provides the method wherein the aromatase inhibitor administered in combination with entinostat is letrozole. Another embodiment provides the method wherein the aromatase inhibitor administered in combination with entinostat is anastrozole. Another embodiment provides the method wherein the aromatase inhibitor administered in combination with entinostat is exemestane.

[0088] Another embodiment provides the method wherein entinostat and the aromatase inhibitor are administered sequentially in either order or simultaneously. Another embodiment provides the method wherein entinostat and the aromatase inhibitor are administered simultaneously. Another embodiment provides the method wherein the aromatase inhibitor is administered first. Another embodiment provides the method wherein the aromatase inhibitor is administered daily and the entinostat is administered periodically. Another embodiment provides the method wherein entinostat is adminsistered weekly and the aromatase inhibitor is administered daily. Another embodiment provides the method wherein entinostat is introduced to an ongoing aromatase inhibitor course of therapy.

[0089] Another embodiment provides the method further comprising administering to the subject one or more therapies in addition to the combination of entinostat and the aromatase inhibitor selected from the group consisting of: letrozole, anastrozole or exemestane, or their pharmaceutically acceptable salts, solvates, or prodrugs.

[0090] Another embodiment provides the method wherein the one or more therapies comprise one or more of radiation therapy, chemotherapy, high dose chemotherapy with stem cell transplant, and monoclonal antibody therapy. Another embodiment provides the method wherein radiation therapy comprises internal and/or external radiation therapy. Another embodiment provides the method wherein the chemotherapy comprisies administering to the subject one or more of doxorubicin, cyclophosphamide, paclitaxel, lapatinib, capecitabine, trastuzumab, bevacizumab, gemcitabine, eribulin, or nab-paclitaxel.

[0091] One embodiment provides a method of treating breast cancer in a patient in need thereof, comprising oral administration of exemestane and entinostat, wherein the entinostat is administered to a fasting patient. Another embodiment provides the method of treating breast cancer wherein the entinostat Tmax is less than 1 hour post administration. Another embodiment provides the method of treating breast cancer wherein the entinostat Tmax is less than 90 minutes post administration. Another embodiment provides the method of treating breast cancer wherein the entinostat Tmax is less than 2 hours post administration. Another embodiment provides the method of treating breast cancer wherein the entinostat Tmax is between 30 minutes and 2 hours post administration. Another embodiment provides the method of treating breast cancer wherein the entinostat Cmax is at least 150 ng/mL following oral administration of entinostat. Another embodiment provides the method of treating breast cancer wherein the entinostat Cmax is at least 125 ng/mL following oral administration of entinostat. Another embodiment provides the method of treating breast cancer wherein the entinostat Cmax is at least 100 ng/mL following oral administration of entinostat.

Another embodiment provides the method of treating breast cancer wherein the entinostat Cmax is at least 80 ng/mL following oral administration of entinostat. Another embodiment provides the method of treating breast cancer wherein the entinostat Cmax is at least 50 ng/mL following oral administration of entinostat. Another embodiment provides the method of treating breast cancer wherein about 5 mg of entinostat is administered. Another embodiment provides the method of treating breast cancer wherein about 10 mg of entinostat is administered. Another embodiment provides the method of treating breast cancer wherein from about 1 mg to about 20 mg of entinostat is administered. Another embodiment provides the method of treating breast cancer wherein entinostat is administered once per week. Another embodiment provides the method of treating breast cancer wherein entinostat is administered for a 28-day cycle. Another embodiment provides the method of treating breast cancer wherein the patient has not consumed food within 2 hours prior to administration of entinostat. Another embodiment provides the method of treating breast cancer wherein the patient has not consumed food within 1 hour prior to administration of entinostat.

[0092] Another embodiment provides the method of treating breast cancer wherein the patient does not consume food within 2 hours after administration of entinostat. Another embodiment provides the method of treating breast cancer wherein the patient does not consume food within 30 minutes after administration of entinostat. Another embodiment provides the method of treating breast cancer wherein the exemestane is administered at a different time of day than entinostat. Another embodiment provides the method of treating breast cancer wherein exemestane is administered after a meal. Another embodiment provides the method of treating breast cancer wherein exemestane is administered with a meal. Another embodiment provides the method of treating breast cancer wherein about 25 mg of exemestane is administered once daily.

Treatment of Lung Cancer

Epidermal Growth Factor Receptor

[0093] In the last few years, knowledge about molecular mechanisms and cellular transformation in association with cancer behavior has increased. More interest has been generated since the development of specific targeted therapies against the processes involved in the carcinogenesis of many types of cancers. During the 1990s it was discovered that the epidermal growth factor receptor (EGFR) played an important role in tumoral biology and behavior. EGFR stimulation activates intracellular signaling and cascades that influence cellular proliferation and mobilization, angiogenesis and other mechanisms. Normal cells are influenced by external factors, in tumor cells it was found that the activation of cell proliferation mediated by this receptor would no longer need external stimuli, but act independently and autonomously. In the case of NSCLC, it was shown that the over-expression of this receptor, as well as specific somatic mutations occurred in their

intracellular domain with tyrosine kinase activity (between exons 18 and 21), which may influence prognosis, being significantly related to stage, survival and chemotherapy response. These data led to the development and study of various substances, including monoclonal antibodies directed to the extracellular domain of EGFR (e.g., cetuximab, Erbitux®) and small molecules that inhibit the tyrosine kinase intracellular domain (tyrosine kinase inhibitors, TKIs) of EGFR (e.g., gefitinib and erlotinib). Preliminary results of randomized clinical trials conducted with these TKIs have shown that their use in patients with advanced disease is effective, significantly increasing the survival of these patients, especially if they harbor mutations in the EGFR which are more frequently found in a subgroup of non-smoking, female patients, of Asian ethnicity and with adenocarcinoma histological sub-type (especially in the presence of bronchioloalveolar carcinoma). Some of these results were so impressive that this phenomenon was designated, the Lazarus effect, and led to the approval, in the United States and Europe, of erlotinib for the second- and third-line treatment of NSCLC patients; and gefitinib in Europe, for patients harboring the EGFR mutation (del Mello, et al., World J Clinc Oncol, Vol. 2, p. 367 (2011)).

[0094] EGFR, also known as ErbB1 or Her1, is a transmembrane glycoprotein encoded by a gene located on chromosome 7 (7p12.1-12.3). EGFR comprises 1186 amino acids (a.a.) and 26 exons. Exons 1-14 encode the extracellular domain, exon 15 encodes the transmembrane region and exons 16-26 the intracellular domain. This glycoprotein belongs to the ErbB receptor family, which also consists of: ErbB2 (HER2/neu), ErbB3 (HER3) and ErbB4 (HER4). Each of these proteins is structurally composed of an extracellular domain, a hydrophobic transmembrane domain and an intracellular domain with intrinsic tyrosine kinase (TK) activity (except ErbB3). These receptors exist as inactive monomers, being activated by their interaction, through the extracellular domain, with growth factors of the EGF family. The binding of ErbB receptor molecules to one of these ligands leads to its interaction with other monomers of the same family (receptor dimerization). This dimerization can occur between two identical receptors (homodimerization, e.g., ErbB1-ErbB1) or between two different receptors (heterodimerization, e.g., ErbB1-ErbB3). The stimulation caused by a specific ligand triggers a unique pattern of dimerization, which is also specific to the tissue/tumor in which the phenomenon occurs. Dimerization of the receptors leads to their autophosphorylation with activation of TK and activation of a cascade of intracellular biochemical processes that regulate such diverse activities, like proliferation, differentiation, apoptosis and cell migration.

E-cadherin

[0095] Epithelial cadherin (E-cadherin), also known as cadherin-1, CAM 120/80 or uvomorulin, is a protein that in humans is encoded by the CDH1 gene. E-cadherin is a classical member of the

cadherin superfamily. E-cadherin is a calcium-dependent cell-cell adhesion glycoprotein composed of five extracellular cadherin repeats (EC1-EC5) in the extracellular domain, a transmembrane domain, an intracellular domain that binds p120-catenin and beta-catenin, and a highly conserved cytoplasmic tail. The intracellular domain contains a highly-phosphorylated region vital to beta-catenin binding and, therefore, to E-cadherin function. Beta-catenin can also bind to alpha-catenin. Alpha-catenin participates in regulation of actin-containing cytoskeletal filaments. In epithelial cells, E-cadherin-containing cell-to-cell junctions are often adjacent to actin-containing filaments of the cytoskeleton.

[0096] Mutations in this gene are correlated with gastric, breast, colorectal, thyroid, and ovarian cancers. Loss of function or expression is thought to contribute to progression in cancer and metastasis. E-cadherin downregulation decreases the strength of cellular adhesion within a tissue, resulting in an increase in cellular motility. This in turn may allow cancer cells to cross the basement membrane and invade surrounding tissues.

Methods for determining E-cadherin levels

[0097] E-cadherin protein levels can be quantitatively measured by ELISA. Some E-cadherin ELISA kits, such as the E-cadherin EIA kit provided by TaKaRA, are a solid phase sandwich EIA that utilizes two mouse monoclonal E-cadherin antibodies (one of which is coated on the plate, and the other is POD-labeled) for detection of human E-cadherin using a two-step incubation method. In the first step, samples are incubated in the antibody-coated microtiter plate. During the second step, the plate is washed and incubated with the POD-labeled E-cadherin antibody. A substrate is added, and the reaction between POD and the substrate (H2O2, TMBZ) results in a color development. The amount of sample soluble E-cadherin is determined by measuring absorbance using an EIA plate reader. Accurate soluble E-cadherin sample concentrations can be determined by comparing their specific absorbances with the absorbance obtained for the Standard plotted on a standard curve. In some embodiments, E-cadherin protein levels are quantitatively measured by ELISA.

[0098] E-cadherin protein levels can be detected by immunohistochemistry. To detect E-cadherin levels in immersion fixed cells, cells are incubated with Human E-Cadherin Antigen Affinity-purified Polyclonal Antibody (R&D Systems® Catalog # AF648) at 10 µg/mL for 3 hours at room temperature. Cells are then stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (R&D Systems® Catalog # NL001) and counterstained with DAPI. E-cadherin and DAPI can be visualized using a fluorescence microscope and filter sets appropriate for the label used. In some embodiments, E-cadherin protein levels are detected by immunhistochemistry.

[0099] E-cadherin protein levels can be detected by immunocytochemistry. Coverslips for immunocytochemistry (ICC) can be prepared using gelatin. In some embodiments, a method for preparing coverslips for ICC includes a) placing sterilized coverslips into the wells of a 24-well plate, b) adding 400 µL of the gelatin-coating solution and c) incubating the coverslips for 10 minutes at room temperature. Then the gelatin-coating solution is removed and the coverslips are air-dried for 15 minutes. The dried coverslips can be stored at room temperature until use. Once the coverslips have been prepared, the cells can be prepared and fixed as follows. Culture cells by adding 500 µL of culture media containing approximately 5000 cells to the wells of a cell culture plate containing gelatin-coated coverslips. When cells have reached the desired density/age, remove the culture media from each well and wash twice with PBS. Add 300-400 µL of 2-4% Formaldehyde Fixative Solution to each well, and incubate for 20 minutes at room temperature. Wash the wells twice with PBS and cover with 400 µL of wash buffer. The coverslips can be stored at 2-8 °C for up to 3 months or they may be stained immediately. Once the cells have been prepared, the cells can be stained for ICC as follows. Wash the coverslips containing the fixed cells two times in 400 µL of wash buffer. Block non-specific staining by adding 400 µL of blocking buffer and incubate for 45 minutes at room temperature. Remove blocking buffer. No rinsing is necessary. Dilute the unconjugated primary antibody (or fluorescence-conjugated primary) in dilution buffer according to the manufacturer's instructions. For fluorescent ICC staining of cells on coverslips using R&D Systems antibodies, it is recommended to incubate at room temperature for 1 hour. Alternatively, incubate overnight at 2-8 °C. Wash two times in 400 µL of wash buffer. If using a primary antibody with a direct fluorescent conjugate, go to step 8. Dilute the secondary antibody in dilution buffer according to the manufacturer's instructions. Add 400 µL to the wells, and incubate at room temperature for 1 hour in the dark. From this step forward samples should be protected from light. Rinse two times in 400 µL of wash buffer. Add 300 µL of the diluted DAPI solution to each well, and incubate 2-5 minutes at room temperature. DAPI binds to DNA and is a convenient nuclear counterstain. It has an absorption maximum at 358 nm and fluoresces blue at an emission maximum of 461 nm. Rinse once with PBS and once with water. Carefully remove the coverslips from the wells and blot to remove any excess water. Dispense 1 drop of anti-fade mounting medium onto the microscope slide per coverslip. Mount the coverslip with the cells facing towards the microscope slide. Visualize using a fluorescence microscope and filter sets appropriate for the label used. Slides can also be stored in a slide box at < -20 °C for later examination. In some embodiments, E-cadherin protein levels are detected by immuncytochemistry.

[00100] E-cadherin gene expression can be determined by measuring E-cadherin methylation. E-cadherin methylation kits, such as the CpG WIZ® E-cadherin amplification kit provided by Millipore®, determine the methylation of status of the E-cadherin promoter by methylation-specific PCR (MSP). The kit contains primers targeted to regions of the promoter where the sequences are most divergent after bisulfite treatment. PCR parameters have been identified so that all primer sets in the kit amplify under the same conditions. Control genomic DNA samples (methylated and unmethylated) for E-cadherin are also included. In some embodiments, E-cadherin gene expression is determined by measuring E-cadherin methylation.

[00101] One embodiment provides a method of treating cancer in an EGFR inhibitor-naïve patient progressed on prior therapy, wherein said patient exhibits high E-cadherin expression levels, the method comprising administering to the patient a combination comprising entinostat and an EGFR inhibitor. Another embodiment provides the method wherein high E-cadherin expression levels are characterized by ELISA, immunohistochemistry, immunocytochemistry or determination of E-cadherin methylation levels. Another embodiment provides the method wherein high E-cadherin expression levels are determined by immunohistochemistry. Another embodiment provides the method wherein the high E-cadherin expression levels are scored as +3 as determined by immunohistochemistry.

Lung Cancer

[00102] Lung cancer is the leading cause of cancer deaths in women and men both in the United States and throughout the world. Lung cancer has surpassed breast cancer as the leading cause of cancer deaths in women. In the United States in 2010, 157,300 people were projected to die from lung cancer, which is more than the number of deaths from colon and rectal, breast, and prostate cancer combined. Only about 2% of those diagnosed with lung cancer that has spread to other areas of the body are alive five years after the diagnosis, although the survival rates for lung cancers diagnosed at the earliest stage are higher, with approximately 49% surviving for five years or longer.

[00103] Cancer occurs when normal cells undergo a transformation that causes them to grow and multiply without control. The cells form a mass or tumor that differs from the surrounding tissues from which it arises. Tumors are dangerous because they take oxygen, nutrients, and space from healthy cells and because they invade and destroy or reduce the ability of normal tissues to function.

[00104] Most lung tumors are malignant. This means that they invade and destroy the healthy tissues around them and can spread throughout the body. The tumors can spread to nearby lymph nodes or through the bloodstream to other organs. This process is called metastasis. When

lung cancer metastasizes, the tumor in the lung is called the primary tumor, and the tumors in other parts of the body are called secondary tumors or metastatic tumors.

[00105] Some tumors in the lung are metastatic from cancers elsewhere in the body. The lungs are a common site for metastasis. If this is the case, the cancer is not considered to be lung cancer. For example, if prostate cancer spreads via the bloodstream to the lungs, it is metastatic prostate cancer (a secondary cancer) in the lung and is not called lung cancer.

[00106] Lung cancer comprises a group of different types of tumors. Lung cancers usually are divided into two main groups that account for about 95% of all cases. The division into groups is based on the type of cells that make up the cancer. The two main types of lung cancer are characterized by the cell size of the tumor when viewed under the microscope. They are called small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC includes several subtypes of tumors. SCLCs are less common, but they grow more quickly and are more likely to metastasize than NSCLCs. Often, SCLCs have already spread to other parts of the body when the cancer is diagnosed. About 5% of lung cancers are of rare cell types, including carcinoid tumor, lymphoma, and others. As used herin, the term “lung cancer” includes, but is not limited to, SCLC, NSCLC, carcinoid tumor, lymphoma, and their various subtypes.

Non-small cell lung cancer

[00107] NSCLC is a cancer of the lung which is not of the small cell carcinoma (oat cell carcinoma) type. The term "non-small cell lung cancer" applies to the various types of bronchogenic carcinomas (those arising from the lining of the bronchi). Examples of specific types of NSCLC include, but are not limited to, adenocarcinoma, squamous cell carcinoma, and large cell cancer (i.e., large cell undifferentiated carcinoma).

[00108] Adenocarcinoma is a cancer that develops in the lining or inner surface of an organ. Adenocarcinoma is the most common type of lung cancer, making up 30%-40% of all cases of lung cancer. A subtype of adenocarcinoma is called bronchoalveolar cell carcinoma, which creates a pneumonia-like appearance on chest X-rays.

[00109] Squamous cell carcinoma is a cancer that begins in squamous cells. Squamos cells are thin, flat cells that look under the microscope like fish scales. Squamous cells are found in the tissue that forms the surface of the skin, the lining of hollow organs of the body, and the passages of the respiratory and digestive tracts. Squamous cell carcinomas may arise in any of these tissues. Squamous cell carcinoma is the second most common type of lung cancer, making up about 30% of all cases.

[00110] Large cell carcinoma shows no evidence of squamous or glandular maturation. Thus these tumors are often diagnosed by default, when all other possibilities have been excluded.

These tumors lack any diagnostic features to suggest their diagnosis prior to biopsy. They tend to grow rapidly, metastasize early, and are strongly associated with smoking. Large cell tumors are usually large, bulky, well-circumscribed, pink-grey masses with extensive hemorrhage and necrosis. Although they commonly have central necrosis, they rarely cavitate. They tend to present in the mid to peripheral lung zones. They may extend locally to involve the segmental or subsegmental bronchi. A variant of large cell carcinoma is giant cell carcinoma. This subtype is particularly aggressive and carries a very poor prognosis. These tumors generally present as a large peripheral mass with a focal necrotic component. They do not involve the large airways, unless by direct extension. Large cell cancer makes up 10%-20% of all cases of lung cancer.

Small cell lung cancer

[00111] SCLC is also called oat cell lung cancer and is a type of lung cancer in which the cells appear small and round under the microscope. SCLC is considered distinct from other lung cancers because of their clinical and biologic characteristics. Small cell lung cancer exhibits aggressive behavior, with rapid growth, early spread to distant sites, exquisite sensitivity to chemotherapy and radiation, and frequent association with distinct paraneoplastic syndromes. Small cell carcinomas arise in peribronchial locations and infiltrate the bronchial submucosa. Widespread metastases occur early in the course of the disease, with common spread to the mediastinal lymph nodes, liver, bones, adrenal glands, and brain. In addition, production of various peptide hormones leads to a wide range of paraneoplastic syndromes; the most common of these is the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) and the syndrome of ectopic adrenocorticotropic hormone (ACTH) production. In addition, autoimmune phenomena may lead to various neurologic syndromes, such as Lambert-Eaton syndrome. SCLC makes up 20% of all cases.

Carcinoid tumor

[00112] Carcinoid tumor is a tumor which secretes large amounts of the hormone serotonin. Carcinoid tumor is also called an argentaffinoma. The tumor usually arises in the gastrointestinal tract, anywhere between the stomach and the rectum (the favorite spot is in the appendix) and from there may metastasize to the liver. In the liver the tumor produces and releases large quantities of serotonin into the systemic bloodstream. The consequences are called the carcinoid syndrome. It is directly due to the serotonin and includes flushing and blushing, swelling of the face (especially around the eyes), flat angiomas (little collections of dilated blood vessels) on the skin, diarrhea, bronchial spasm, rapid pulse, low blood pressure and tricuspid and pulmonary stenosis (narrowing of the tricuspid and pulmonic valves of the heart), often with regurgitation. One or more of four kinds of treatment are used for carcinoid tumors: surgery (to take out the cancer);

radiation therapy (using high-dose x-rays to kill the cancer cells); biological therapy (using the body's natural immune system to fight the cancer); and chemotherapy (using drugs to kill cancer cells). Carcinoid tumors are considered a type of endocrine tumor since they secrete a hormone (serotonin). They can occur as part of certain genetic disorders such as the multiple endocrine neoplasia (MEN) type 1 and neurofibromatosis type 1 (NF1 or von Recklinghausen disease). Carcinoid tumors account for 1% of all cases.

Lymphoma

[00113] Lymphoma is a type of cancer involving cells of the immune system, called lymphocytes, and primarily represents cells involved in the lymphatic system of the body. Lymphoma is a malignant transformation of either B or T cells or their subtypes. Lymphomas fall into one of two major categories: Hodgkin's lymphoma (HL, previously called Hodgkin's disease) and all other lymphomas (non-Hodgkin's lymphomas or NHLs). These two types occur in the same places, may be associated with the same symptoms, and often have similar appearance on physical examination. However, they are readily distinguishable via microscopic examination. Hodgkin's disease develops from a specific abnormal B lymphocyte lineage. NHL may derive from either abnormal B or T cells and are distinguished by unique genetic markers. There are five subtypes of Hodgkin's disease and about 30 subtypes of non-Hodgkin's lymphoma. Because there are so many different subtypes of lymphoma, the classification of lymphomas is complicated (it includes both the microscopic appearance as well as genetic and molecular markers). Many of the NHL subtypes look similar, but they are functionally quite different and respond to different therapies with different probabilities of cure. HL subtypes are microscopically distinct, and typing is based upon the microscopic differences as well as extent of disease.

EGFR inhibitors

[00114] EGFR inhibitors interrupt signaling through the epiderdermal growth factor receptor (EGFR) in target cells. Certain EGFR inhibitors, such as erlotinib, have been approved for the treatment of metastatic NSCLC. For advanced NSCLC, EGFR inhibitors, such as gefitinib, have been approved. Several more EGFR inhibitors are being tested in clinical trials for the treatment of NSCLC and additional lung cancers.

[00115] As described herein, an “EGFR inhibitor” is a molecule which inhibits the activity of the EGF receptor. Compounds which are inhibitors of EGFR can be readily identified by one skilled in the art using methods such as, for example, an EGFR kinase assay which measures ADP formed from a kinase reaction.

[00116] Inhibition of EGFR as a treatment option for lung cancer has been studied with some success. Currently three EGFR inhibitors, erlotinib, gefinitib, and cetuximab, are approved for marketing in the US for the treatment of lung cancer.

[00117] Erlotinib (Tarceva ®) is approved to treat metastatic non-small cell lung cancer and pancreatic cancer that cannot be removed by surgery or has metastasized. This small-molecule drug inhibits the tyrosine kinase activity of EGFR.

[00118] Gefitinib (Iressa®) is approved to treat patients with advanced non-small cell lung cancer. This small-molecule drug is restricted to use in patients who, in the opinion of their treating physician, are currently benefiting, or have previously benefited, from gefitinib treatment. Gefitinib inhibits the tyrosine kinase activity of the epidermal growth factor receptor (EGFR), which is overproduced by many types of cancer cells.

[00119] Cetuximab (Erbitux ®) is a monoclonal antibody that is approved for treating some patients with squamous cell carcinoma of the head and neck or colorectal cancer. The therapy binds to the external portion of EGFR, thereby preventing the receptor from being activated by growth signals, which may inhibit signal transduction and lead to antiproliferative effects.

[00120] Additional examples of EGFR inhibitors include, but are not limited to, panitumumab, vandetanib, lapatinib, canertinib, afatinib, necitumumab, nimotuzumab, PF299804, RO5083945, ABT-806, and AP26113.

[00121] Panitumumab (Vectibix ®) is approved to treat some patients with metastatic colon cancer. This monoclonal antibody attaches to EGFR and prevents it from sending growth signals.

[00122] Vandetanib (Caprelsa ®) is approved to treat patients with metastatic medullary thyroid cancer who are ineligible for surgery. This small-molecule drug binds to and blocks the growth-promoting activity of several tyrosine kinase enzymes, including EGFR, several receptors for vascular endothelial growth factor receptor (VEGF), and RET.

[00123] Lapatinib (Tykerb ®) is approved for the treatment of certain types of advanced or metastatic breast cancer. This small-molecule drug inhibits several tyrosine kinases, including the tyrosine kinase activity of HER-2. Lapatinib treatment prevents HER-2 signals from activating cell growth.

[00124] Canertinib is an orally bioavailable irreversible pan-ErbB tyrosine kinase inhibitor, targeting EGFR, HER-2, ErbB-3 and ErbB-4. It effectively inhibits the growth of esophageal squamous cell carcinoma which co-expresses both EGFR and HER2 with the inhibition of phosphorylation of both MAPK and AKT. In vitro studies of human cancer cell lines indicate that canertinib results in prompt, potent, and sustained inhibition of tyrosine kinase activity.

[00125] Afatinib is an irreversible EGFR/HER2. In cell-free in vitro kinase assays, afatinib shows potent activity against wild-type and mutant forms of EGFR and HER2, similar to gefitinib in potency for L858R EGFR, but about 100-fold more active against the gefitinib resistant L858R-T790M EGFR double mutant. Afatinib was effective in inhibiting survival of lung cancer cell lines harboring wild-type (H1666) or L858R/T790M (NCI-H1975) EGFR. Assessed in a standard xenograft model of the epidermoid carcinoma cell line A431. Daily oral treatment with afatinib at 20 mg/kg for 25 days resulted in dramatic tumor regression with a cumulative treated/control tumor volume ratio (T/C ratio) of 2%. Like lapatinib and neratinib, afatinib is a next generation tyrosine kinase inhibitor (TKI) that irreversibly inhibits human epidermal growth factor receptor 2 (Her2) and epidermal growth factor receptor (EGFR) kinases. Afatinib is not only active against EGFR mutations targeted by first generation TKIs like erlotinib or gefitinib, but also against those not sensitive to these standard therapies. Because of its additional activity against Her2, it is investigated for breast cancer as well as other EGFR and Her2 driven cancers.

[00126] Necitumumab is a fully human IgG1 monoclonal antibody directed against the epidermal growth factor receptor (EGFR) with potential antineoplastic activity. Necitumumab binds to and blocks the ligand binding site of EGFR, thereby preventing the activation and subsequent dimerization of the receptor. This may lead to an inhibition of EGFR-dependent downstream pathways and so inhibition of EGFR-dependent tumor cell proliferation and metastasis.

[00127] Nimotuzumab is a humanized monoclonal antibody directed against the epidermal growth factor receptor (EGFR) with potential antineoplastic activity. Nimotuzumab binds to and inhibits EGFR, resulting in growth inhibition of tumor cells that overexpress EGFR. This agent may act synergistically with radiation therapy.

[00128] PF299804 is a potent, irreversible inhibitor of human epidermal growth factor receptor (HER)-1/EGFR, -2, and -4 tyrosine kinases (TK), is active in E-sensitive and -resistant preclinical models. PF299804 had clinical activity in phase I/II trials in EGFR TK inhibitor (TKI)-refractory NSCLC.

[00129] RO5083945 is a glycoengineered anti EGFR IgG1 mAb exhibiting increased binding affinity for all FcγRIIIa variants expressed on immune effector cells. RO5083945 demonstrates significantly improved cell killing in ADCC-based assays and greater activity in in vivo models compared to cetuximab and panitumumab. Hence, RO5083945 has the potential to show clinical activity in patients with solid tumors, including KRAS mutant CRC.

[00130] ABT-806 is a humanized monoclonal antibody (MoAb) against human epidermal growth factor receptor (EGFR) with antineoplastic activity. MoAb ABT-806 targets the EGFR

deletion variant, de2-7 EGFR as well as wild-type EGFR expressed in cells overexpressing the receptor, thereby preventing the activation and subsequent dimerization of the receptor; the decrease in receptor activation and dimerization result in an inhibition in signal transduction and anti-proliferative effects. This MoAb targets cells expressing aberrant EGFR, hence making it an ideal candidate for generation of radioisotope or toxin conjugates.

[00131] AP26113 is an orally available inhibitor of receptor tyrosine kinases anaplastic lymphoma kinase (ALK) and the epidermal growth factor receptor (EGFR) with potential antineoplastic activity. Dual ALK/EGFR inhibitor AP26113 binds to and inhibits ALK kinase and ALK fusion proteins as well as EGFR and mutant forms. This leads to the inhibition of ALK kinase and EGFR kinase, disrupts their signaling pathways and eventually inhibits tumor cell growth in susceptible tumor cells. In addition, AP26113 appears to overcome mutation-based resistance. ALK belongs to the insulin receptor superfamily and plays an important role in nervous system development; ALK dysregulation and gene rearrangements are associated with a series of tumors. EGFR is overexpressed in a variety of cancer cell types.

Additional Therapy

[00132] Available additional treatments for lung cancer that may be advantageously employed in combination with the therapies disclosed herein include, without limitation, radiation therapy, chemotherapy, antibody therapy, and tyrosine kinase inhibitors as adjuvant therapy.

[00133] Radiation therapy is a cancer treatment that uses high-energy x-rays or other types of radiation to kill cancer cells or keep them from growing. Chemotherapy is a cancer treatment that uses drugs to stop the growth of cancer cells, either by killing the cells or by stopping them from dividing. When chemotherapy is taken by mouth or injected into a vein or muscle, the drugs enter the bloodstream and can reach cancer cells throughout the body (systemic chemotherapy). When chemotherapy is placed directly into the spinal column, an organ, or a body cavity such as the abdomen, the drugs mainly affect cancer cells in those areas (regional chemotherapy). The way the chemotherapy is given depends on the type and stage of the cancer being treated.

[00134] Different chemotherapeutic agents are known in the art for treating lung cancer. Cytoxic agents used for treating lung cancer include carboplatin (for example, Paraplatin®, Paraplat®), cisplatin (for example, Platinol®, Platinol-Aq®), crizotinib (for example Xalkori®), etoposide (for example Toposar®, VePesid®), etoposide Phosphate (for example Etopophos®), gemcitabine hydrochloride (for example Gemzar®), gemcitabine-cisplatin, methotrexate (for example Abitrexate®, Folex®, Folex Pfs®, Methotrexate Lpf®, Mexate®, Mexate-Aq®), paclitaxel (for example Taxol®), pemetrexed Disodium (for example Alimta®), and topotecan Hydrochloride (for example Hycamtin®)

[00135] Monoclonal antibody therapy is a cancer treatment that uses antibodies made in the laboratory, from a single type of immune system cell. These antibodies can identify substances on cancer cells or normal substances that may help cancer cells grow. The antibodies attach to the substances and kill the cancer cells, block their growth, or keep them from spreading. Monoclonal antibodies are given by infusion. They may be used alone or to carry drugs, toxins, or radioactive material directly to cancer cells. Monoclonal antibodies are also used in combination with chemotherapy as adjuvant therapy.

[00136] Bevacizumab (Avastin®) is a recombinant humanized monoclonal antibody directed against the vascular endothelial growth factor (VEGF), a pro-angiogenic cytokine. Bevacizumab binds to VEGF and inhibits VEGF receptor binding, thereby preventing the growth and maintenance of tumor blood vessels. Bevacizumab is used currently to treat several types of cancer, including certain types of colorectal, lung, breast, and kidney cancers and glioblastoma.

[00137] Additional, illustrative, treatments that may be advantageously combined with the compositions and therapies disclosed herein may include, without limitation, administration of agents including, but not limited to lapatinib, alone or in combination with capecitabine, docetaxel, epirubicin, epothilone A, B or D, goserelin acetate, paclitaxel, pamidronate, bevacizumab, or trastuzumab.

[00138] In some embodiments, the additional therapy comprises chemotherapy comprising administering to the subject one or more of doxorubicin, cyclophosphamide, paclitaxel, lapatinib, capecitabine, trastuzumab, bevacizumab, gemcitabine, eribulin, or nab-paclitaxel.

Methods for the Treatment of Lung Cancer

[00139] One embodiment provides a method of treating cancer in an EGFR inhibitor-naïve patient progressed on prior therapy, wherein the method comprises: (1) determining the E-cadherin expression level in the patient; (2) selecting the patient exhibiting a high E-cadherin expression level scored as +3; and (3) administering to the patient a combination comprising entinostat and an EGFR inhibitor. In some instances, entinostat is administered to a fasting patient.

[00140] In some embodiments, the Cmax is higher when entinostat is administered without food as compared to the Cmax when entinostat is administered with food. In some embodiments, the ratio of Cmax following administration of entinostat under fasting conditions to Cmax following administration of entinostat under fed conditions is at least about 2:1. In one embodiment, the ratio of Cmax following administration of entinostat under fasting conditions to Cmax following administration of entinostat under fed conditions is at least about 3:1. In one embodiment, the ratio of Cmax following administration of entinostat under fasting conditions to Cmax following administration of entinostat under fed conditions is at least about 4:1. In one

embodiment, the ratio of Cmax following administration of entinostat under fasting conditions to Cmax following administration of entinostat under fed conditions is at least about 5:1. In one embodiment, the ratio of Cmax following administration of entinostat under fasting conditions to Cmax following administration of entinostat under fed conditions is at least about 6:1. In one embodiment, the ratio of Cmax following administration of entinostat under fasting conditions to Cmax following administration of entinostat under fed conditions is at least about 7:1.

[00141] In some embodiments, the Tmax is lower when entinostat is administered without food as compared to the Tmax when entinostat is administered with food. In some embodiments, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 2:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 3:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 4:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 5:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 6:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 7:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 8:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 9:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 10:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 11:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 12:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 13:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 14:1. In one embodiment, the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 15:1.

[00142] In some embodiments, the EGFR inhibitor is administered a different time of day than entinostat. In one embodiment, the EGFR inhibitor is administerd to a fasting patient.

[00143] Another embodiment provides the method wherein the prior therapy was one prior chemotherapy.

[00144] Another embodiment provides the method wherein the prior therapy was two or more prior chemotherapies.

[00145] Another embodiment provides the method wherein high E-cadherin expression levels are determined by ELISA, immunohistochemistry, immunocytochemistry or determination of E-cadherin methylation levels. Another embodiment provides the method wherein high E-cadherin expression levels are determined by immunohistochemistry. Another embodiment provides the method wherein the high E-cadherin expression levels are scored as +3 as determined by immunohistochemistry.

[00146] Another embodiment provides the method wherein the cancer is lung cancer.

[00147] Another embodiment provides the method wherein the lung cancer is non-small cell lung cancer.

[00148] Another embodiment provides the method wherein the EGFR inhibitor administered in combination with entinostat is erlotinib.

[00149] Another embodiment provides the method wherein entinostat and the EGFR inhibitor are administered sequentially in either order or simultaneously. Another embodiment provides the method wherein entinostat and the EGFR inhibitor are administered simultaneously. Another embodiment provides the method wherein the EGFR inhibitor is administered first.

[00150] Another embodiment provides the method wherein the EGFR inhibitor is administered daily and the entinostat is administered periodically. Another embodiment provides the method wherein the EGFR inhibitor is administered daily and the entinostat is administered weekly.

[00151] Another embodiment provides a method of treating cancer in an EGFR inhibitor-naïve patient progressed on prior therapy, wherein said patient exhibits high E-cadherin expression levels, the method comprising administering to the patient a combination comprising entinostat and an EGFR inhibitor.

[00152] Another embodiment provides the method of treating cancer in an EGFR inhibitor-naïve patient progressed on prior therapy, wherein said patient exhibits high E-cadherin expression levels, wherein the method further comprises administering to the subject one or more additional therapies in addition to the combination of entinostat and the EGFR inhibitor. Another embodiment provides the method wherein the one or more therapies comprise one or more of radiation therapy, chemotherapy, high dose chemotherapy with stem cell transplant, and monoclonal antibody therapy. Another embodiment provides the method wherein radiation therapy comprises internal

and/or external radiation therapy. Another embodiment provides the method wherein the chemotherapy comprises administering to the subject one or more of doxorubicin, cyclophosphamide, paclitaxel, lapatinib, capecitabine, trastuzumab, bevacizumab, gemcitabine, eribulin, or nab-paclitaxel. Another embodiment provides the method wherein the chemotherapy comprises administering to the subject one or more IGF-1R inhibitors. Another embodiment provides the method wherein the IGF-1R inhibitor is AEW541.

[00153] One embodiment provides a method of treating non-small cell lung cancer in a patient in need thereof, comprising oral administration of erlotinib and entinostat, wherein the entinostat is administered to a fasting patient. Another embodiment provides the method of treating non-small cell lung cancer wherein the entinostat Tmax is less than 1 hour post administration. Another embodiment provides the method of treating non-small cell lung cancer wherein the entinostat Tmax is less than 90 minutes post administration. Another embodiment provides the method of treating non-small cell lung cancer wherein the entinostat Tmax is less than 2 hours post administration. Another embodiment provides the method of treating non-small cell lung cancer wherein the entinostat Tmax is between 30 minutes and 2 hours post administration. Another embodiment provides the method of treating non-small cell lung cancer wherein the entinostat Cmax is at least 150 ηg/mL following oral administration of entinostat. Another embodiment provides the method of treating non-small cell lung cancer wherein the entinostat Cmax is at least 125 ηg/mL following oral administration of entinostat. Another embodiment provides the method of treating non-small cell lung cancer wherein the entinostat Cmax is at least 100 ηg/mL following oral administration of entinostat. Another embodiment provides the method of treating non-small cell lung cancer wherein the entinostat Cmax is at least 80 ηg/mL following oral administration of entinostat. Another embodiment provides the method of treating non-small cell lung cancer wherein the entinostat Cmax is at least 50 ηg/mL following oral administration of entinostat. Another embodiment provides the method of treating non-small cell lung cancer wherein about 10 mg of entinostat is administered. Another embodiment provides the method of treating non-small cell lung cancer wherein from about 1 mg to about 20 mg of entinostat is administered. Another embodiment provides the method of treating non-small cell lung cancer wherein entinostat is administered every 14 days. Another embodiment provides the method of treating non-small cell lung cancer wherein the entinostat is administered for a month. Another embodiment provides the method of treating non-small cell lung cancer wherein the patient has not consumed food within 2 hours prior to administration of entinostat. Another embodiment provides the method of treating non-small cell lung cancer wherein the patient has not consumed food within 1 hour prior to administration of entinostat. Another embodiment provides the method of treating non-small cell

lung cancer wherein the patient does not consume food within 1 hour after administration of entinostat. Another embodiment provides the method of treating non-small cell lung cancer wherein the patient does not consume food within 30 minutes after administration of entinostat. Another embodiment provides the method of treating non-small cell lung cancer wherein the erlotinib is administered at a different time of day than entinostat. Another embodiment provides the method of treating non-small cell lung cancer wherein the erlotinib is administered once daily to the fasting patient. Another embodiment provides the method of treating non-small cell lung cancer wherein the patient has not consumed food within 2 hours prior to administration of erlotinib. Another embodiment provides the method of treating non-small cell lung cancer wherein the patient does not consume food within 1 hour after administration of erlotinib. Another embodiment provides the method of treating non-small cell lung cancer wherein about 150 mg of erlotinib is administered.

Oral Formulations

[00154] Oral formulations containing the active pharmaceutical ingredients described herein may comprise any conventionally used oral forms, including: tablets, capsules, pills, troches, lozenges, pastilles, cachets, pellets, medicated chewing gum, granules, bulk powders, effervescent or non-effervescent powders or granules, solutions, emulsions, suspensions, solutions, wafers, sprinkles, elixirs, syrups, buccal forms, and oral liquids. Capsules may contain mixtures of the active compound(s) with inert fillers and/or diluents such as the pharmaceutically acceptable starches (e.g. corn, potato or tapioca starch), sugars, artificial sweetening agents, powdered celluloses, such as crystalline and microcrystalline celluloses, flours, gelatins, gums, etc. Useful tablet formulations may be made by conventional compression, wet granulation or dry granulation methods and utilize pharmaceutically acceptable diluents, binding agents, lubricants, disintegrants, surface modifying agents (including surfactants), suspending or stabilizing agents, including, but not limited to, magnesium stearate, stearic acid, talc, sodium lauryl sulfate, microcrystalline cellulose, carboxymethylcellulose calcium, polyvinylpyrrolidone, gelatin, alginic acid, acacia gum, xanthan gum, sodium citrate, complex silicates, calcium carbonate, glycine, dextrin, sucrose, sorbitol, dicalcium phosphate, calcium sulfate, lactose, kaolin, mannitol, sodium chloride, talc, dry starches and powdered sugar. In some embodiments are surface modifying agents which include nonionic and anionic surface modifying agents. For example, surface modifying agents include, but are not limited to, poloxamer 188, benzalkonium chloride, calcium stearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, magnesium aluminum silicate, and triethanolamine. Oral formulations herein may utilize standard delay or time release formulations to alter the absorption of the active

compound(s). The oral formulation may also consist of administering the active ingredient in water or a fruit juice, containing appropriate solubilizers or emulsifiers as needed.

Oral Administration

[00155] As described herein, the combination therapy described herein can be given simultaneously or can be given in a staggered regimen, with entinostat being given at a different time during the course of chemotherapy than the EGFR inhibitor. This time differential may range from several minutes, hours, days, weeks, or longer between administrations of the two compounds. Therefore, the term combination does not necessarily mean administered at the same time or as a unitary dose, but that each of the components are administered during a desired treatment period. The agents may also be administered by different routes. As is typical for chemotherapeutic regimens, a course of chemotherapy may be repeated several weeks later, and may follow the same timeframe for administration of the two compounds, or may be modified based on patient response.

[00156] In other embodiments, the pharmaceutical compositions provided herein may be provided in solid, semisolid, or liquid dosage forms for oral administration. As used herein, oral administration also include buccal, lingual, and sublingual administration. Suitable oral dosage forms include, but are not limited to, tablets, capsules, pills, troches, lozenges, pastilles, cachets, pellets, medicated chewing gum, granules, bulk powders, effervescent or non-effervescent powders or granules, solutions, emulsions, suspensions, solutions, wafers, sprinkles, elixirs, and syrups. In addition to the active ingredient(s), the pharmaceutical compositions may contain one or more pharmaceutically acceptable carriers or excipients, including, but not limited to, binders, fillers, diluents, disintegrants, wetting agents, lubricants, glidants, coloring agents, dye-migration inhibitors, sweetening agents, and flavoring agents.

[00157] Binders or granulators impart cohesiveness to a tablet to ensure the tablet remaining intact after compression. Suitable binders or granulators include, but are not limited to, starches, such as corn starch, potato starch, and pre-gelatinized starch (e.g., STARCH 1500); gelatin; sugars, such as sucrose, glucose, dextrose, molasses, and lactose; natural and synthetic gums, such as acacia, alginic acid, alginates, extract of Irish moss, Panwar gum, ghatti gum, mucilage of isabgol husks, carboxymethylcellulose, methylcellulose, polyvinylpyrrolidone (PVP), Veegum, larch arabogalactan, powdered tragacanth, and guar gum; celluloses, such as ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose, methyl cellulose, hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC), hydroxypropyl methyl cellulose (HPMC); microcrystalline celluloses, such as AVICEL-PH-101, AVICEL-PH-103, AVICEL RC-581, AVICEL-PH-105 (FMC Corp., Marcus Hook, PA); and mixtures thereof. Suitable fillers

include, but are not limited to, talc, calcium carbonate, microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder or filler may be present from about 50 to about 99% by weight in the pharmaceutical compositions provided herein.

[00158] Suitable diluents include, but are not limited to, dicalcium phosphate, calcium sulfate, lactose, sorbitol, sucrose, inositol, cellulose, kaolin, mannitol, sodium chloride, dry starch, and powdered sugar. Certain diluents, such as mannitol, lactose, sorbitol, sucrose, and inositol, when present in sufficient quantity, can impart properties to some compressed tablets that permit disintegration in the mouth by chewing. Such compressed tablets can be used as chewable tablets.

[00159] Suitable disintegrants include, but are not limited to, agar; bentonite; celluloses, such as methylcellulose and carboxymethylcellulose; wood products; natural sponge; cation-exchange resins; alginic acid; gums, such as guar gum and Veegum HV; citrus pulp; cross-linked celluloses, such as croscarmellose; cross-linked polymers, such as crospovidone; cross-linked starches; calcium carbonate; microcrystalline cellulose, such as sodium starch glycolate; polacrilin potassium; starches, such as corn starch, potato starch, tapioca starch, and pre-gelatinized starch; clays; aligns; and mixtures thereof. The amount of disintegrant in the pharmaceutical compositions provided herein varies upon the type of formulation, and is readily discernible to those of ordinary skill in the art. The pharmaceutical compositions provided herein may contain from about 0.5 to about 15% or from about 1 to about 5% by weight of a disintegrant.

[00160] Suitable lubricants include, but are not limited to, calcium stearate; magnesium stearate; mineral oil; light mineral oil; glycerin; sorbitol; mannitol; glycols, such as glycerol behenate and polyethylene glycol (PEG); stearic acid; sodium lauryl sulfate; talc; hydrogenated vegetable oil, including peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil; zinc stearate; ethyl oleate; ethyl laureate; agar; starch; lycopodium; silica or silica gels, such as AEROSIL® 200 (W.R. Grace Co., Baltimore, MD) and CAB-O-SIL® (Cabot Co. of Boston, MA); and mixtures thereof. The pharmaceutical compositions provided herein may contain about 0.1 to about 5% by weight of a lubricant.

[00161] Suitable glidants include colloidal silicon dioxide, CAB-O-SIL® (Cabot Co. of Boston, MA), and asbestos-free talc. Coloring agents include any of the approved, certified, water soluble FD&C dyes, and water insoluble FD&C dyes suspended on alumina hydrate, and color lakes and mixtures thereof. A color lake is the combination by adsorption of a water-soluble dye to a hydrous oxide of a heavy metal, resulting in an insoluble form of the dye. Flavoring agents include natural flavors extracted from plants, such as fruits, and synthetic blends of compounds which produce a pleasant taste sensation, such as peppermint and methyl salicylate. Sweetening

agents include sucrose, lactose, mannitol, syrups, glycerin, and artificial sweeteners, such as saccharin and aspartame. Suitable emulsifying agents include gelatin, acacia, tragacanth, bentonite, and surfactants, such as polyoxyethylene sorbitan monooleate (TWEEN® 20), polyoxyethylene sorbitan monooleate 80 (TWEEN® 80), and triethanolamine oleate. Suspending and dispersing agents include sodium carboxymethylcellulose, pectin, tragacanth, Veegum, acacia, sodium carbomethylcellulose, hydroxypropyl methylcellulose, and polyvinylpyrolidone. Preservatives include glycerin, methyl and propylparaben, benzoic add, sodium benzoate and alcohol. Wetting agents include propylene glycol monostearate, sorbitan monooleate, diethylene glycol monolaurate, and polyoxyethylene lauryl ether. Solvents include glycerin, sorbitol, ethyl alcohol, and syrup. Examples of non-aqueous liquids utilized in emulsions include mineral oil and cottonseed oil. Organic acids include citric and tartaric acid. Sources of carbon dioxide include sodium bicarbonate and sodium carbonate.

[00162] It should be understood that many carriers and excipients may serve several functions, even within the same formulation.

[00163] In further embodiments, the pharmaceutical compositions provided herein may be provided as compressed tablets, tablet triturates, chewable lozenges, rapidly dissolving tablets, multiple compressed tablets, or enteric-coating tablets, sugar-coated, or film-coated tablets. Enteric-coated tablets are compressed tablets coated with substances that resist the action of stomach acid but dissolve or disintegrate in the intestine, thus protecting the active ingredients from the acidic environment of the stomach. Enteric-coatings include, but are not limited to, fatty acids, fats, phenylsalicylate, waxes, shellac, ammoniated shellac, and cellulose acetate phthalates. Sugar-coated tablets are compressed tablets surrounded by a sugar coating, which may be beneficial in covering up objectionable tastes or odors and in protecting the tablets from oxidation. Film-coated tablets are compressed tablets that are covered with a thin layer or film of a water-soluble material. Film coatings include, but are not limited to, hydroxyethylcellulose, sodium carboxymethylcellulose, polyethylene glycol 4000, and cellulose acetate phthalate. Film coating imparts the same general characteristics as sugar coating. Multiple compressed tablets are compressed tablets made by more than one compression cycle, including layered tablets, and press-coated or dry-coated tablets.

[00164] The tablet dosage forms may be prepared from the active ingredient in powdered, crystalline, or granular forms, alone or in combination with one or more carriers or excipients described herein, including binders, disintegrants, controlled-release polymers, lubricants, diluents, and/or colorants. Flavoring and sweetening agents are especially useful in the formation of chewable tablets and lozenges.

[00165] The pharmaceutical compositions provided herein may be provided as soft or hard capsules, which can be made from gelatin, methylcellulose, starch, or calcium alginate. The hard gelatin capsule, also known as the dry-filled capsule (DFC), consists of two sections, one slipping over the other, thus completely enclosing the active ingredient. The soft elastic capsule (SEC) is a soft, globular shell, such as a gelatin shell, which is plasticized by the addition of glycerin, sorbitol, or a similar polyol. The soft gelatin shells may contain a preservative to prevent the growth of microorganisms. Suitable preservatives are those as described herein, including methyl- and propyl-parabens, and sorbic acid. The liquid, semisolid, and solid dosage forms provided herein may be encapsulated in a capsule. Suitable liquid and semisolid dosage forms include solutions and suspensions in propylene carbonate, vegetable oils, or triglycerides. Capsules containing such solutions can be prepared as described in U.S. Pat. Nos. 4,328,245; 4,409,239; and 4,410,545. The capsules may also be coated as known by those of skill in the art in order to modify or sustain dissolution of the active ingredient.

[00166] In other embodiments, the pharmaceutical compositions provided herein may be provided in liquid and semisolid dosage forms, including emulsions, solutions, suspensions, elixirs, and syrups. An emulsion is a two-phase system, in which one liquid is dispersed in the form of small globules throughout another liquid, which can be oil-in-water or water-in-oil. Emulsions may include a pharmaceutically acceptable non-aqueous liquids or solvent, emulsifying agent, and preservative. Suspensions may include a pharmaceutically acceptable suspending agent and preservative. Aqueous alcoholic solutions may include a pharmaceutically acceptable acetal, such as a di(lower alkyl) acetal of a lower alkyl aldehyde (the term "lower" means an alkyl having between 1 and 6 carbon atoms), e.g., acetaldehyde diethyl acetal; and a water-miscible solvent having one or more hydroxyl groups, such as propylene glycol and ethanol. Elixirs are clear, sweetened, and hydroalcoholic solutions. Syrups are concentrated aqueous solutions of a sugar, for example, sucrose, and may also contain a preservative. For a liquid dosage form, for example, a solution in a polyethylene glycol may be diluted with a sufficient quantity of a pharmaceutically acceptable liquid carrier, *e.g.*, water, to be measured conveniently for administration.

[00167] Other useful liquid and semisolid dosage forms include, but are not limited to, those containing the active ingredient(s) provided herein, and a dialkylated mono- or poly-alkylene glycol, including, 1,2-dimethoxymethane, diglyme, triglyme, tetraglyme, polyethylene glycol-350-dimethyl ether, polyethylene glycol-550-dimethyl ether, polyethylene glycol-750-dimethyl ether, wherein 350, 550, and 750 refer to the approximate average molecular weight of the polyethylene glycol. These formulations may further comprise one or more antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate, vitamin E, hydroquinone,

hydroxycoumarins, ethanolamine, lecithin, cephalin, ascorbic acid, malic acid, sorbitol, phosphoric acid, bisulfite, sodium metabisulfite, thiodipropionic acid and its esters, and dithiocarbamates.

[00168] The pharmaceutical compositions provided herein for oral administration may be also provided in the forms of liposomes, micelles, microspheres, or nanosystems. Miccellar dosage forms can be prepared as described in U.S. Pat. No. 6,350,458.

[00169] In other embodiments, the pharmaceutical compositions provided herein may be provided as non- effervescent or effervescent, granules and powders, to be reconstituted into a liquid dosage form. Pharmaceutically acceptable carriers and excipients used in the non-effervescent granules or powders may include diluents, sweeteners, and wetting agents. Pharmaceutically acceptable carriers and excipients used in the effervescent granules or powders may include organic acids and a source of carbon dioxide.

[00170] Coloring and flavoring agents can be used in all of the above dosage forms.

[00171] The pharmaceutical compositions provided herein may be formulated as immediate or modified release dosage forms, including delayed-, sustained, pulsed-, controlled, targeted-, and programmed-release forms.

[00172] In further embodiments, the pharmaceutical compositions provided herein may be co-formulated with other active ingredients which do not impair the desired therapeutic action, or with substances that supplement the desired action.

EXAMPLES

Example 1 A Phase 1, Randomized, Open-Label Study to Assess the Food Effect on the Pharmacokinetics of Entinostat in Postmenopausal Women with Locally Recurrent or Metastatic ER+ Breast Cancer and Men and Women with Progressive Non-Small Cell Lung Cancer

Protocol

[00173] Title: A Phase 1 Study to Assess the Food Effect on the Pharmacokinetics of Entinostat in Postmenopausal Women with Locally Recurrent or Metastatic ER+ Breast Cancer and Men and Women with Progressive Non-Small Cell Lung Cancer

Study Phase: Phase 1

Indication: Breast cancer; non-small cell lung cancer

Primary Objective:

• To evaluate the effect of food on the pharmacokinetics of entinostat in women with breast cancer and men and women with non-small cell lung cancer (NSCLC).

Secondary Objective:

• Safety: To evaluate the safety and tolerability of entinostat in combination with exemestane or erlotinib as measured by adverse events, laboratory parameters and electrocardiac assessments

Exploratory Objectives:

• To determine food effect on degree of protein lysine acetylation changes induced by entinostat administration

• To evaluate the degree of acetylation in patients receiving entinostat as related to entinostat plasma concentrations and treatment duration.

• To determine if addition of exemestane or erlotinib effects degree of acetylation changes induced by entinostat administration

Study Design:

[00174] This is Phase 1, randomized, open-label, two-period, two-sequence cross-over study of entinostat. Patients will be randomized in a 1:1 ratio to receive entinostat 10 mg with or without food on Cycle 1 Day 1 (C1D1). Patients randomized to receive entinostat with food on C1D1 will receive a second dose of entinostat 10 mg without food on Cycle 1 Day 15 (C1D15). Similarly, patients randomized to receive entinostat without food on C1D1 will receive a second dose of entinostat 10 mg with food on C1D15. The randomization will be stratified by sex. Each cycle in the study will be for 28 days duration. Blood samples will be obtained pre-dose and serial blood samples will be taken after each dose to assess pharmacokinetics. In addition, blood samples will be drawn for assessment of entinostat acetylation.

[00175] For Cycle 2 and all subsequent cycles, all patients will continue to receive 10 mg entinostat on Days 1 and 15 of each cycle. Those with breast cancer will also receive exemestane orally (po) 25 mg once daily (qd) starting on Cycle 2 Day 1. Those with NSCLC will also receive 150 mg po erlotinib qd starting on Cycle 2 Day 1.

[00176] Patients will be assessed at screening and at pre-prescribed times during study enrollment using standard clinical and laboratory assessments. Patents will also be assessed for tumor response after each 2 cycles. Tumor progression will be assessed by CT, MRI or other appropriate radiologic study. Patients will continue receiving their appropriate cycles of study treatment until tumor progression or adverse events occur which necessitate discontinuing therapy as determined by the Investigator.

Endpoints:

Primary Pharmacokinetic Endpoints

• Cmax, maximum plasma concentration

• Tmax, time of maximum plasma concentration

• AUClast, area under the plasma concentration-time curve from time zero to the last measurable concentration

[00177] AUCinf, area under the plasma concentration-time curve from time zero extrapolated to infinity via the following AUClast+Clast/λz

• λz, Terminal elimination rate constant

Pharmacodynamic Secondary Endpoints

• Change from baseline in protein lysine acetylation as measured by peripheral blood monocytes

[00178] Safety Endpoints

• Incidence of treatment-emergent adverse events, serious adverse events, adverse events resulting in the permanent discontinuation of study drug, and deaths occurring within 30-days of the last dose of study drug

• Changes from baseline in laboratory, vital signs, and electrocardiogram results

[00179] Sample Size: Up to 28 patients (approximately 14 patients with breast cancer and 14 patients with NSCLC, with a minimum of 4 male patients) will be enrolled to ensure that 24 patents (approximately 12 per treatment sequence) complete Cycle 1 of study treatment.

Summary of Subject Eligibility Criteria: The study will enroll postmenopausal women with histologically or cytologically confirmed estrogen receptor positive (ER+) breast cancer at initial diagnosis whose disease has progressed to where the investigator determines that the patient is a candidate to receive exemestane. In addition, it will enroll adults with cytologically or histologically confirmed NSCLC of stage IIIb or IV who are eligible candidates for erlotinib therapy. All patients must be at least 18 years old, with Eastern Cooperative Oncology Group (ECOG) status of 0 or 1.

Investigational Product:

[00180] Entinostat is a synthetic small molecule with a molecular formula C21H20N4O3 and a molecular weight of 376.41. Entinostat is classified as an antineoplastic agent, specifically functioning as an inhibitor of histone deacetylases by promoting hyperacetylation of nucleosomal histones. Entinostat is orally bioavailable and will be supplied as yellow coated tablets containing 5.0 mg of active ingredient.

Study Treatment:

One cycle will be defined as 28 days of study treatment.

All Patients: Cycle 1 Only

• Group A: Entinostat 10 mg po on Day 1 under test fasted conditions and Day 15 under test fed conditions

• Group B: Entinostat 10 mg po on Day 1 under test fed conditions and Day 15 under test fasted conditions

Breast Cancer Patients Only: Cycle 2 and All Subsequent Cycles

• Exemestane 25 mg will be administered once daily starting on Cycle 2 Day 1.

• Entinostat will be administered at a dose of 10 mg po on Days 1 and 15 at least 2 hours after breakfast, followed by at least a 1-hour fast.

NSCLC Patients Only: Cycle 2 and All Subsequent Cycles

• Erlotinib 150 mg will be administered once daily starting on Cycle 2 Day 1.

• Entinostat will be administered at a dose of 10 mg po on Days 1 and 15, at least 2 hours after breakfast, followed by at least a 1-hour fast.

[00181] Pharmacokinetic Evaluation, All Patients (Study Days 1 and 15 of Cycle 1 only) Patients will be administered one of two treatments according to their randomization: 10 mg entinostat under test fed conditions or 10 mg entinostat under test fasted conditions. All treatments will be given as a single dose with 240 mL of water. Water will be allowed in all treatment groups as desired for up to 2 hours prior to dosing, then restricted up until 2 hours post-dose except for the fluid taken during breakfast in the fed treatment group. Blood will be obtained for determination of entinostat concentrations at the following times: pre-dose (within 60 minutes of dosing), and then at .25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 72, 120, 168, 240, and 336 hours post dose. ECGs will be obtained in triplicate at -60 and -45 minutes pre-dose and then at the same time points as the PK blood samples. Holter monitor will be used for Cycle 1 Day 1 and Day 15 from pre-dose through the 12-hour post dose.

[00182] Pharmacodynamic Evaluation, All Patients

Study Days 1 and 15 of Cycle 1

Blood will be obtained for determination of entinostat acetylation at the following times: pre-dose (within 60 minutes of dosing), and then at 12, 24, 168 and 336 hours post dose.

Study Day 1 of Cycle 3

Blood will be obtained for determination of protein acetylation pre-dose on Day 1 of Cycle 3.

End of Study Assessment

Blood will be obtained for determination of protein acetylation at the final study visit.

Study Duration

Patients will continue to receive protocol therapy until progressive disease or unacceptable or intolerable toxicity is encountered.

Statistical Considerations:

[00183] Schuirmann's two one-sided test procedure for interval hypotheses will be used to compare the fed and fasted states for differences in average bioavailability. The difference in average bioavailability will be determined by the extent of exposure of entinostat based on AUC_{last}, and C_{max}.

[00184] The following set of hypotheses will be tested for comparing the fed state to that of the fasted state based on logarithmically transformed data:

$$H_0 : \mu_{Fed}/\mu_{Fasted} \leq 0.80 \text{ or } \mu_{Fed}/\mu_{Fasted} \geq 1.25$$

$$H_1 : 0.80 < \mu_{Fed}/\mu_{Fasted} < 1.25$$

where μ_{Fed} and μ_{Fasted} represent the population mean AUC_{last}, AUC_{inf}, or C_{max} for the fed and fasted conditions, respectively. A total of 24 evaluable patients, 12 per treatment sequence, will be required to detect with 90% power and type 1 error rate of 5%, the aforementioned difference in average bioavailability between the fed and fasted conditions. The intra-patient coefficient of variation (CV) is assumed to be 22%. The true mean ratio between the fed and fasted conditions is assumed to be 1.0.

[00185] Patients will be considered evaluable for PK analysis if they receive entinostat in each treatment period according to the conditions defined by the randomization (ie, fed or fasted). Patients must also have sufficient plasma concentration-time data from each treatment period in order to provide for meaningful assessment of the PK parameters (eg, Cmax, AUClast).

[00186] Individual patient concentration-time data will be listed and displayed graphically on the linear and log scale. The concentration-time data will be summarized descriptively in tabular and graphical format (linear and log scale). PK parameters estimated using noncompartmental methods will be calculated using WinNonlin version 5.1 or higher. Such estimates will be listed and summarized descriptively in tabular and graphical format.

[00187] For selected PK parameters (eg, Cmax, AUClast), comparisons between the fed and fasted conditions will be made using a linear mixed effects ANOVA model. The model will include terms for treatment (fed, fasted), period, and sequence as fixed effects, and patient within sequence as the random effect. The assessment of bioequivalence will be based on the classical (shortest) confidence interval approach which is operationally equivalent to Schuirmann's two one-sided test procedure for interval hypotheses. The estimates from the ANOVA model will be used to calculate 90% confidence limits for the ratio of the true mean AUC for the fed and fasted conditions. Bioequivalence in average bioavailability will be concluded if the 90% confidence interval for the ratio (back transformed) is wholly contained within the equivalence limits of 80% and 125%.

[00188] Time to maximum observed plasma concentration of entinostat (Tmax) will be summarized for the fed and fasted conditions using descriptive statistics and graphical displays. Individual patient differences for Tmax between the fed and fasted condition will be calculated; symmetric nonparametric confidence interval for the median difference will be provided. Inferential comparison of Tmax between the fed and fasted condition will be made using Wilcoxon's signed rank test.

[00189] Changes in protein lysine acetylation (primary pharmacodynamic parameter for this study) will be analyzed in the same or similar manner as described above for the PK analysis. Joint analysis of PK by acetylation may also be performed.

[00190] Safety data analysis will be conducted on all patients receiving at least one dose of entinostat. Analyses will consist of data summaries for clinical and laboratory parameters, and for adverse events. Unless otherwise specified, the safety analyses will be performed by primary diagnosis. The number and percentage of patients with one or more adverse events will be summarized by relationship to the individual study treatments and by severity grade. Severity grade will be determined using the NCI-CTCAE (version 4.0). Adverse events will be coded using the Medical Dictionary for Regulatory Activities Terminology (MedDRA). Laboratory parameters will be summarized using descriptive statistics, by shifts relative to baseline, and data listings of clinically significant abnormalities. Vital signs and ECG data will be summarized by changes from baseline values using descriptive statistics.

Results

[00191] The results of the food effect study are presented herein. Figure 1 displays the mean concentration time profiles following administration of 10 mg entinostat under fasted or fed conditions. A summary of the pharmacokinetic parameters is presented in Table 1.

[00192] Coadministration of 10 mg entinostat with food results in a lag in drug absorption and a delay in Tmax (median tmax= 0.76 hrs under fasted conditions; median tmax=11 hrs under fed conditions). A significant reduction in maximum drug concentrations (71% decrease in Cmax) was observed. Overall exposure, as estimated by AUClast and AUCinf, was reduced by approximately 15-17% when entinostat was administered with a high fat meal. The mean elimination half-life of entinostat was estimated as 140 hrs under fasted conditions and 178 hrs under fed conditions. There was a high degree of variability in the half-life estimates for the fed group (%CV=70%), likely due to the small sample size. A few individuals did have a significantly prolonged t1/2 value in the fed group. Median values for t1/2 suggest that the two groups are comparable.

[00193] Conclusion: A food effect is evident for entinostat when it is co-administered with a high fat meal, resulting in a delayed tmax and a reduced Cmax and AUC.

Example 2

[00194] A Method for Treating Postmenopausal Women With Locally Recurrent or Metastatic Estrogen Receptor-Positive Breast Cancer by Administering Entinostat and a Non-Steroidal Aromatase Inhibitor, Exemestane

[00195] The purpose of this study is to evaluate the safety and efficacy of entinostat in combination with exemestane in the treatment of advanced breast cancer.

[00196] Primary Outcome Measures are to compare the efficacy of exemestane alone with exemestane plus entinostat, as determined by the duration of progression free survival (PFS) measured from the date of randomization.

[00197] Secondary Outcome Measures are to compare objective response rate (ORR) and clinical benefit rate (CBR), and to evaluate the safety and tolerability of entinostat in combination with exemestane as measured by adverse events and laboratory safety parameters.

[00198] Study Design

Arm	Assigned Interventions
1: Experimental exemestane (Aromasin) 25mg daily plus entinostat 5mg PO once/week Interventions: • Drug: entinostat • Drug: exemestane	Drug: entinostat entinostat 5mg tablet PO once/week without food Drug: exemestane exemestane 25mg PO QD Other Name: Aromasin
2: Placebo Comparator exemestane (Aromasin) 25mg daily plus placebo PO once/week Intervention: Drug: exemestane	Drug: exemestane exemestane 25mg PO QD Other Name: Aromasin

Eligibility Criteria

Ages Eligible for Study: 18 Years and older
Genders Eligible for Study: Female
Accepts Healthy Volunteers: No

Inclusion Criteria:

- Postmenopausal female patients
- Histologically or cytologically confirmed ER+ breast cancer

- Relapsed or progressed on prior treatment with AI
- Metastatic disease must be measurable
- Patients receiving palliative radiation at the non-target lesions must have a 2 week wash out period following completion of the treatment prior to enrollment
- Patient may have had one prior chemotherapy as part of first line therapy as long as it was received before initiation of prior AI
- ECOG performance status: 0 to 1
- Laboratory parameters: a)Hemoglobin $\geq$ 9.0 g/dL; platelets $\geq$ 100.0 x 109/L; ANC $\geq$ 1.5 x 109/L without the use of hematopoietic growth factors b)Creatinine less than 2.5 times the upper limit of normal for the institution c)AST and ALT less than 2.5 times the upper limit of normal for the institution
- Able to understand and give written informed consent and comply with study procedures

Exclusion Criteria:

- Relapse on treatment with non-steroidal AI after less than 12 months for patients in the adjuvant setting
- Progressive disease after less than 3 months treatment with most recent AI for patients with metastatic disease
- Rapidly progressive, life-threatening metastases
- Any palliative radiotherapy to the measurable lesion
- Previous treatment with entinostat or any other HDAC inhibitor including valproic acid
- Allergy to benzamides or inactive components of the study drug
- A history of allergies to any active or inactive ingredients of exemestane
- Any concomitant medical condition that precludes adequate study treatment compliance
- Patient is currently enrolled in (or completed within 30 days before study drug administration) another investigational drug study
- Patient is currently receiving treatment with valproic acid, Zolinza(vorinostat) or any other HDAC inhibitor or DNA methyltransferase inhibitor or any systemic anticancer treatment (with the exception of Lupron)

Example 3

[00199] A Method for Treating Patients With Non-Small Cell Lung Carcinoma Who Are Progressing on Erlotinib by Administering a Combination of Erlotinib and Entinostat

[00200] Primary Outcome Measures:

Disease control rate (complete response, partial response, or stable disease for at least 3 months)

[00201] Secondary Outcome Measures:

Progression-free survival rate at 2 months

Progression-free survival rate at 4 months

[00202] Study Design

Arm	Assigned Interventions
1: Experimental "Erlotinib-responsive" patients are those who progressed following either a complete or partial response to erlotinib or a period of stable disease lasting at least 3 months. Interventions: Drug: entinostat Drug: erlotinib	Drug: entinostat entinostat (10 mg fixed dose PO Q2W) on days 1 and 15 of a 28-day cycle for up to 6 cycles without food Drug: erlotinib erlotinib (150 mg PO QD) for up to six (6) 28-day cycles
2: Experimental "Erlotinib-nonresponsive" patients are those who either progressed immediately during treatment with erlotinib (i.e. after at least 1 full cycle of erlotinib treatment) or had an objective response or period of stable disease lasting less than 3 months. Interventions: Drug: entinostat Drug: erlotinib	Drug: entinostat entinostat (10 mg fixed dose PO Q2W) on days 1 and 15 of a 28-day cycle for up to 6 cycles without food Drug: erlotinib erlotinib (150 mg PO QD) for up to six (6) 28-day cycles

Eligibility Criteria

Ages Eligible for Study: 18 Years and older

Genders Eligible for Study: Both

Accepts Healthy Volunteers: No

Inclusion Criteria:

- Cytologically or histologically confirmed NSCLC of stage IIIb (pleural effusion) or IV
- Disease is progressing (either no response to treatment or subsequent relapse after an objective response) on erlotinib treatment, based on at least 2 scans (the last being within

4 weeks of study enrollment and can serve as the baseline scan for the patient's screening into the study)

- Recovered from any toxicity associated with the most recent cancer treatment (no greater than grade 1 toxicity on CTCAE scale or to prior baseline condition)
- At least 1 measurable lesion ≥ 20mm by conventional CT scan or ≥ 10mm by spiral CT scan
- ECOG performance score of 0, 1, or 2 and life expectancy of at least 3 months
- Paraffin-embedded tumor specimen available for correlative studies
- Male or female over 18 years of age
- Hemoglobin ≥ 9.0 g/dL; platelets ≥ 75 x 10^9/L; ANC ≥ 1.0 x 10^9/L without the use of hematopoietic growth factors
- Coagulation tests within the normal range
- Bilirubin and creatinine less than 2 times the upper limit of normal for the institution
- AST and ALT less than 3 times the upper limit of normal for the institution
- Potassium, magnesium and phosphorus within the normal range for the institution (supplementation is permissible)
- Willing to use accepted and effective methods of contraception during the study (both men and women as appropriate) and for 3 months after the last dose of entinostat
- Patient or legally acceptable representative has granted written informed consent before any study-specific procedure (including special screening tests) is performed

Exclusion Criteria:

- Prior stem cell transplant
- Symptomatic CNS involvement
- Prior treatment with an HDAC inhibitor
- Concurrent anticancer therapy, with the exception of radiotherapy for a non-target study lesion
- Currently taking medication(s) on the prohibited medication list
- Systemic chemotherapy or treatment with an investigational agent within 28 days before enrollment
- Current use of valproic acid
- Untreated or unstable brain metastases, or taken steroids for this condition within 4 weeks of study drug administration

- Currently active second malignancy, or any malignancy within the last 5 years other than cured basal or squamous cell skin carcinoma, cervical carcinoma in situ, or superficial bladder cancer
- Inability to swallow oral medications or a gastrointestinal malabsorption condition
- Uncontrolled infection requiring IV antibiotics, antivirals, or antifungals, known HIV infection, or active hepatitis B or C infection
- Abnormal cardiac function as defined as clinically significant findings on ECG (multifocal PVCs, ST-T wave changes consistent with myocardial infarction or acute ischemia, QTc greater than 500 milliseconds), tachycardia, or left ventricular ejection fraction less than 40% on MUGA scan
- Another serious or uncontrolled medical condition within 3 months of enrollment such as hypertension, diabetes mellitus, or suppressed immune system
- Known hypersensitivity to benzamides
- Morbid obesity
- Women who are currently pregnant or breast-feeding
- Patient is currently enrolled in (or completed within 28 days) another investigational drug study
- Patient unavailable for on-study or follow-up assessments
- Patient has any kind of medical, psychiatric, or behavioral disorder that places the patient at increased risk for study participation or compromises the ability of the patient to give written informed consent and/or to comply with study procedures and requirement

Table 1: Summary of pharmacokinetic parameters following administration of 10 mg entinostat under fasted and fed conditions

Period		T1/2 (hr)	Tlag (hr)	Tmax (hr)	Cmax (ng/mL)	AUClast (ng*hr/mL)	AUCinf (ng*hr/mL)	Cmax ratio	AUClast ratio	AUCinf ratio
Fasted	N	13	16	16	16	16	13	15	15	9
	Mean	140.007	0	1.598	206.675	2585.481	2722.196	0.29	0.83	0.85
	SD	47.59	0	2.823	99.166	955.476	672.132	0.33	0.11	0.19
	Min	80.06	0	0.5	44.6	1430.01	1733.02	0.08	0.62	0.61
	Median	137.74	0	0.76	211.5	2395.97	2562.72	0.15	0.84	0.85
	Max	250.73	0	12	378	5688.79	4170.88	1.33	0.99	1.27
	CV%	34		176.7	48	37	24.7	113.06	12.75	22.58
Fed	N	10	16	16	16	16	10			
	Mean	177.68	0.047	12.697	42.394	2148.01	2694.917			
	SD	125.47	0.101	8.83	26.279	831.324	1132.392			
	Min	79.33	0	0.5	19.4	1107.15	1214.04			
	Median	124.65	0	11.03	33.35	1979.54	2549.14			
	Max	433.32	0.25	24.93	120	4597.26	4784.52			
	CV%	70.6	215	69.5	62	38.7	42			

Cmax ratio = Cmax Fed/Cmax Fasted
AUClast ratio = AUClast Fed/AUClast Fasted
AUCinf ratio = AUCinf Fed/AUCinf Fasted

CLAIMS

1. A method of treating breast cancer in a patient in need thereof, comprising oral administration of exemestane and entinostat, wherein the entinostat is administered to a fasting patient.

2. The method of claim 1, wherein the entinostat Tmax is less than 1 hour post administration.

3. The method of claim 1, wherein the entinostat Tmax is less than 90 minutes post administration.

4. The method of claim 1, wherein the entinostat Tmax is less than 2 hours post administration.

5. The method of claim 1, wherein the entinostat Tmax is between 30 minutes and 2 hours post administration.

6. The method of any of claims 1-5, wherein the entinostat Cmax is at least 150 ng/mL following oral administration of entinostat.

7. The method of any of claims 1-5, wherein the entinostat Cmax is at least 125 ng/mL following oral administration of entinostat.

8. The method of any of claims 1-5, wherein the entinostat Cmax is at least 100 ng/mL following oral administration of entinostat.

9. The method of any of claims 1-5, wherein the entinostat Cmax is at least 80 ng/mL following oral administration of entinostat.

10. The method of any of claims 1-5, wherein the entinostat Cmax is at least 50 ng/mL following oral administration of entinostat.

11. The method of any of claims 1-10, wherein about 5 mg of entinostat is administered.

12. The method of any of claims 1-10, wherein about 10 mg of entinostat is administered.

13. The method of any of claims 1-10, wherein from about 1 mg to about 20 mg of entinostat is administered.

14. The method of any of claims 1-13, wherein entinostat is administered once per week.

15. The method of any of claims 1-14, wherein entinostat is administered for a 28-day cycle.

16. The method of any of claims 1-15, wherein the patient has not consumed food within 2 hours prior to administration of entinostat.

17. The method of any of claims 1-15, wherein the patient has not consumed food within 1 hour prior to administration of entinostat.

18. The method of any of claims 1-17, wherein the patient does not consume food within 2 hours after administration of entinostat.

19. The method of any of claims 1-17, wherein the patient does not consume food within 30 minutes after administration of entinostat.

20. The method of any of claims 1-19, wherein the exemestane is administered at a different time of day than entinostat.

21. The method of any of claims 1-20, wherein exemestane is administered after a meal.

22. The method of any of claims 1-20, wherein exemestane is administered with a meal.

23. The method of any of claims 1-22, wherein about 25 mg of exemestane is administered once daily.

24. A method of treating non-small cell lung cancer in a patient in need thereof, comprising oral administration of erlotinib and entinostat, wherein the entinostat is administered to a fasting patient.

25. The method of claim 24, wherein the entinostat Tmax is less than 1 hour post administration.

26. The method of claim 24, wherein the entinostat Tmax is less than 90 minutes post administration.

27. The method of claim 24, wherein the entinostat Tmax is less than 2 hours post administration.

28. The method of claim 24, wherein the entinostat Tmax is between 30 minutes and 2 hours post administration.

29. The method of any of claims 24-28, wherein the entinostat Cmax is at least 150 ηg/mL following oral administration of entinostat.

30. The method of any of claims 24-28, wherein the entinostat Cmax is at least 125 ηg/mL following oral administration of entinostat.

31. The method of any of claims 24-28, wherein the entinostat Cmax is at least 100 ηg/mL following oral administration of entinostat.

32. The method of any of claims 24-28, wherein the entinostat Cmax is at least 80 ηg/mL following oral administration of entinostat.

33. The method of any of claims 24-28, wherein the entinostat Cmax is at least 50 ηg/mL following oral administration of entinostat.

34. The method of any of claims 24-33, wherein about 10 mg of entinostat is administered.

35. The method of any of claims 24-33, wherein from about 1 mg to about 20 mg of entinostat is administered.

36. The method of any of claims 24-35, wherein entinostat is administered every 14 days.

37. The method of any of claims 24-36, wherein the entinostat is administered for a month.

38. The method of any of claims 24-37, wherein the patient has not consumed food within 2 hours prior to administration of entinostat.

39. The method of any of claims 24-37, wherein the patient has not consumed food within 1 hour prior to administration of entinostat.

40. The method of any of claims 24-39, wherein the patient does not consume food within 1 hour after administration of entinostat.

41. The method of any of claims 24-39, wherein the patient does not consume food within 30 minutes after administration of entinostat.

42. The method of any of claims 24-41, wherein the erlotinib is administered at a different time of day than entinostat.

43. The method of any of claims 24-42, wherein the erlotinib is administered once daily to the fasting patient.

44. The method of any of claims 24-43, wherein the patient has not consumed food within 2 hours prior to administration of erlotinib.

45. The method of any of claims 24-44, wherein the patient does not consume food within 1 hour after administration of erlotinib.

46. The method of any of claims 24-45, wherein about 150 mg of erlotinib is administered.

47. A method of treating cancer in a patient in need thereof, comprising oral administration of entinostat, wherein the administration of entinostat under fasting conditions results in an increase of the Cmax as compared to the administration of entinostat under fed conditions, and wherein the ratio of Cmax following administration under fasting conditions to Cmax following administration under fed conditions is at least about 2:1.

48. The method of claim 47, wherein the ratio of Cmax following administration under fasting conditions to Cmax following administration under fed conditions is at least about 3:1.

49. The method of claim 47 or claim 48, wherein the ratio of Cmax following administration under fasting conditions to Cmax following administration under fed conditions is at least about 4:1.

50. The method of any of claims 47-49, wherein the ratio of Cmax following administration under fasting conditions to Cmax following administration under fed conditions is at least about 5:1.

51. The method of any of claims 47-50, wherein the ratio of Cmax following administration under fasting conditions to Cmax following administration under fed conditions is at least about 6:1.

52. The method of any of claims 47-51, wherein the ratio of Cmax following administration under fasting conditions to Cmax following administration under fed conditions is at least about 7:1.

53. The method of any of claims 47-52, wherein the cancer is lung cancer.

54. The method of claim 53, wherein the lung cancer is non-small cell lung cancer.

55. The method of any of claims 47-52, wherein the cancer is breast cancer.

56. The method of claim 53 or claim 54, further comprising oral administration of an EGFR inhibitor.

57. The method of claim 56, wherein the EGFR inhibitor is erlotinib.

58. The method of claim 57, wherein the erlotinib is administered at a different time of day than entinostat.

59. The method of claim 57 or claim 58, wherein the patient has not consumed food within 2 hours prior to administration or erlotinib.

60. The method of any of claims 57-59, wherein the patient does not consume food within 1 hour after administration of erlotinib.

61. The method of any of claims 57-60, wherein about 150 mg of erlotinib is administered.

62. The method of any of claims 57-61, wherein the erlotinib is administered once daily.

63. The method of claim 55, further comprising oral administration of an aromatase inhibitor.

64. The method of claim 63, wherein the aromatase inhibitor is exemestane.

65. The method of claim 64, wherein the exemestane is administered at a different time of day than entinostat.

66. The method of claim 64 or claim 65, wherein exemestane is administered after a meal.

67. The method of claim 64 or claim 65, wherein exemestane is administered with a meal.

68. The method of any of claims 64-67, wherein about 25 mg of exemestane is administered.

69. The method of any of claims 64-68, wherein the exemestane is administered once daily.

70. The method of any of claims 47-69, wherein the patient is administered about 10 mg of entinostat.

71. The method of any of claims 47-69, wherein the patient is administered about 5 mg of entinostat.

72. The method of any of claims 47-69, wherein the patient is administered from about 1 mg to about 20 mg of entinostat.

73. The method of any of claims 47-72, wherein the patient has not consumed food within 2 hours prior to administration of entinostat under fasting conditions.

74. The method of any of claims 47-72, wherein the patient has not consumed food within 1 hour prior to administration of entinostat under fasting conditions.

75. The method of any of claims 47-74, wherein the patient does not consume food within 2 hours after administration of entinostat under fasting conditions.

76. The method of any of claims 47-74, wherein the patient does not consume food within 30 minutes after administration of entinostat under fasting conditions.

77. The method of any of claims 47-76, wherein the patient consumes a high fat meal under fed conditions.

78. A method of treating cancer in a patient in need thereof, comprising oral administration of entinostat, wherein the administration of entinostat under fed conditions results in an increase of the Tmax as compared to the administration of entinostat under fasting conditions, and wherein the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is at least about 2:1.

79. The method of claim 78, wherein the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is from about 2:1 to about 5:1.

80. The method of claim 78, wherein the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is from about 5:1 to about 8:1.

81. The method of claim 78, wherein the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is from about 8:1 to about 12:1.

82. The method of claim 78, wherein the ratio of Tmax following administration under fed conditions to Tmax following administration under fasting conditions is from about 12:1 to about 15:1.

83. The method of any of claims 78-82, wherein the cancer is lung cancer.

84. The method of claim 83, wherein the lung cancer is non-small cell lung cancer.

85. The method of any of claims 78-82, wherein the cancer is breast cancer.

86. The method of claim 83 or claim 84, further comprising oral administration of an EGFR inhibitor.

87. The method of claim 86, wherein the EGFR inhibitor is erlotinib.

88. The method of claim 87, wherein the erlotinib is administered at a different time of day than entinostat.

89. The method of claim 87 or claim 88, wherein the patient has not consumed food within 2 hours prior to administration or erlotinib.

90. The method of any of claims 87-89, wherein the patient does not consume food within 1 hour after administration of erlotinib.

91. The method of any of claims 87-90, wherein about 150 mg of erlotinib is administered.

92. The method of any of claims 87-91, wherein the erlotinib is administered once daily.

93. The method of claim 85, further comprising oral administration of an aromatase inhibitor.

94. The method of claim 93, wherein the aromatase inhibitor is exemestane.

95. The method of claim 94, wherein the exemestane is administered at a different time of day than entinostat.

96. The method of claim 94 or claim 95, wherein exemestane is administered after a meal.

97. The method of claim 94 or claim 95, wherein exemestane is administered with a meal.

98. The method of any of claims 94-97, wherein about 25 mg of exemestane is administered.

99. The method of any of claims 94-98, wherein the exemestane is administered once daily.

100. The method of any of claims 78-99, wherein the patient is administered about 10 mg of entinostat.

101. The method of any of claims 78-99, wherein the patient is administered about 5 mg of entinostat.

102. The method of any of claims 78-99, wherein the patient is administered from about 1 mg to about 20 mg of entinostat.

103. The method of any of claims 78-102, wherein the patient has not consumed food within 2 hours prior to administration of entinostat under fasting conditions.

104. The method of any of claims 78-102, wherein the patient has not consumed food within 1 hour prior to administration of entinostat under fasting conditions.

105. The method of any of claims 78-104, wherein the patient does not consume food within 2 hours after administration of entinostat under fasting conditions.

106. The method of any of claims 78-104, wherein the patient does not consume food within 30 minutes after administration of entinostat under fasting conditions.

107. The method of any of claims 78-106, wherein the patient consumes a high fat meal under fed conditions.

108. A method of treating cancer in a patient in need thereof, comprising oral administration of entinostat, wherein the Cmax of entinostat is increased when the entinostat is administered under fasting conditions, compared to when entinostat is administered under fed conditions.

109. A method of treating cancer in a patient in need thereof, comprising oral administration of entinostat, wherein the Tmax of entinostat is increased when the entinostat is administered under fed conditions, compared to when entinostat is administered under fasting conditions.

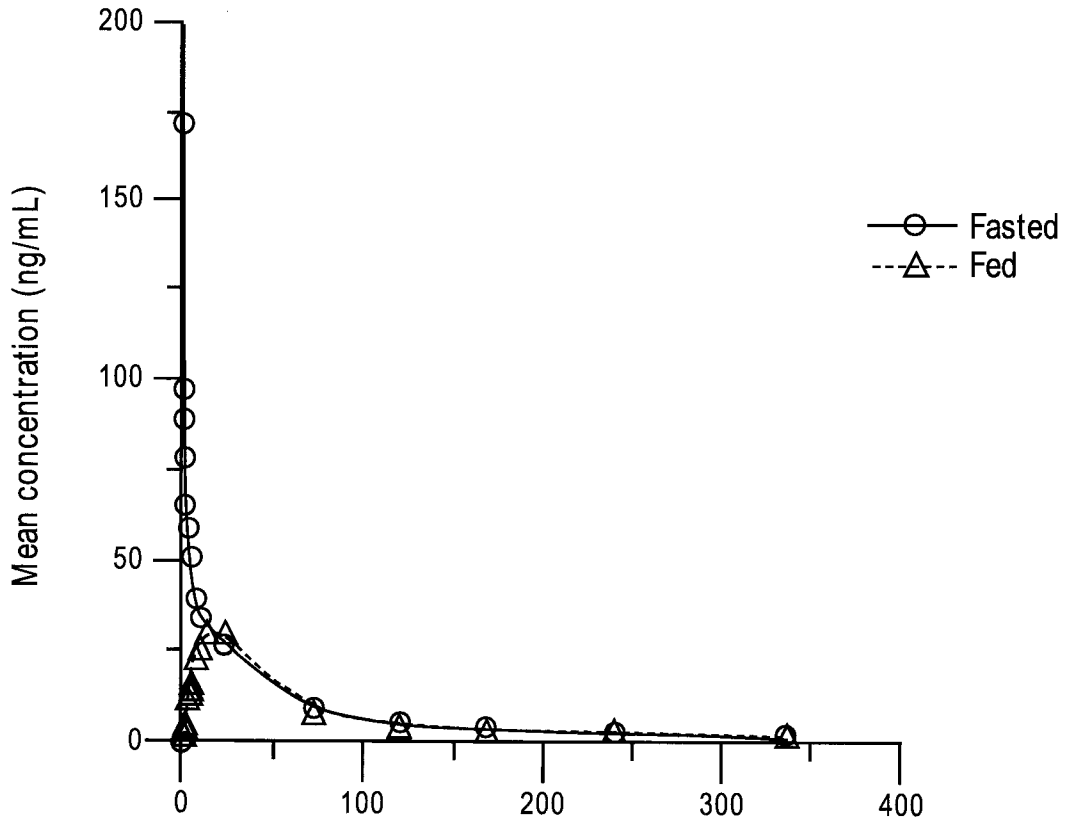

Figure 1(a)

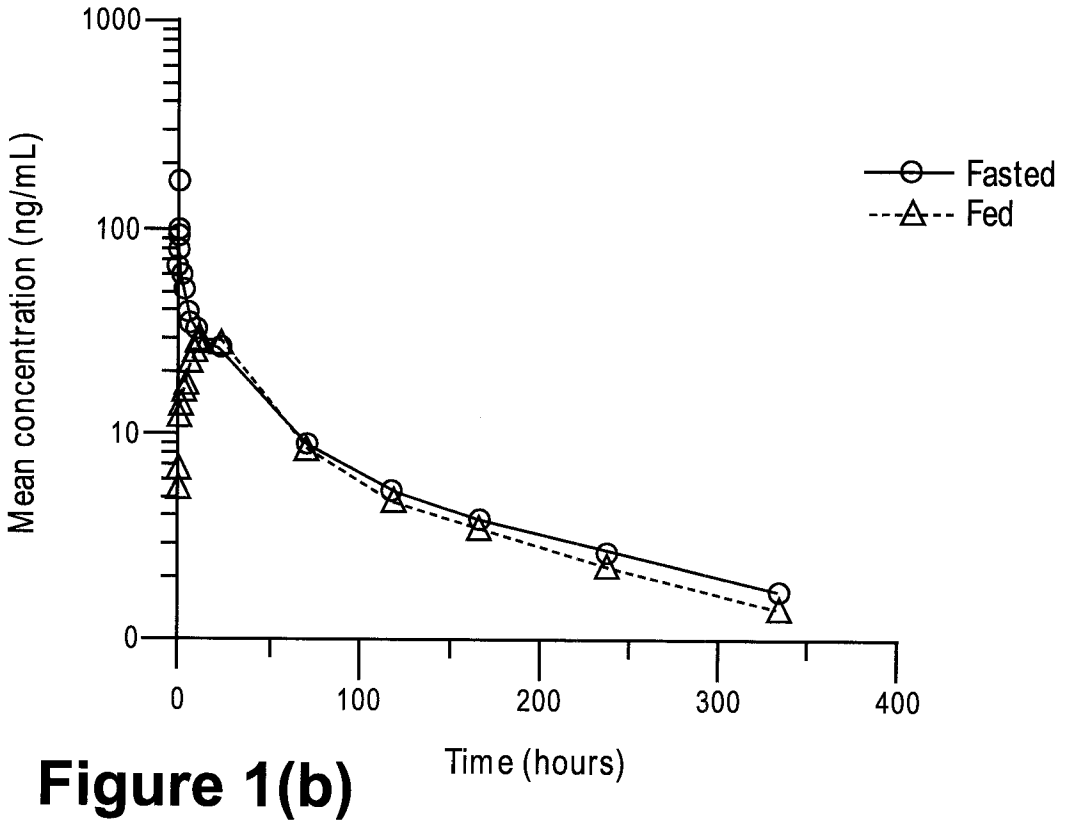

Figure 1(b)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/036651

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K31/4406 (2014.01)
CPC - A61K 31/517
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 31/00, 31/56, 31/4406, 31/5685; A61P 35/00; C07D 239/00 (2014.01)
USPC - 435/375; 514/1, 170, 171, 217.08, 266.1, 357

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 31/517, 31/519, 31/4406, 45/06; A61P 35/00 (2014.06)

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X – Y	ACHARYA et al. "Clinical Pharmacology of MS-275: A Histone Deacetylase Inhibitor," 2005. Virginia Commonwealth University, Pgs. 1-246. Retrieved from the Internet: <scholarscompass.vcu.edu/cgi/viewcontent.cgi?article=1831&context=etd> on 23 August 2014 (23.08.2014). entire document	78-82, 109 --- 83-85
X	DONOVAN et al. "Phase I trial of the oral histone deacetylase inhibitor MS-275 administered with food," Journal of Clinical Oncology, 20 June 2006 (20.06.2012), Vol. 24, No. 18S, Pg. 1. entire document	47-49, 108
Y	WO 2013/033656 A1 (GOODE NOW et al) 07 March 2013 (07.03.2013) entire document	1-10, 85
Y	US 2010/0305167 A1 (BURK et al) 02 December 2010 (02.12.2010) entire document	1-10, 24-33
Y	US 2011/0182888 A1 (ORDENTLICH et al) 28 June 2011 (28.06.2011) entire document	24-33, 83, 84

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 August 2014	05 SEP 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/036651

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

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

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

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

3. ☒ Claims Nos.: 11-23, 34-46, 50-77, 86-107
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.