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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.

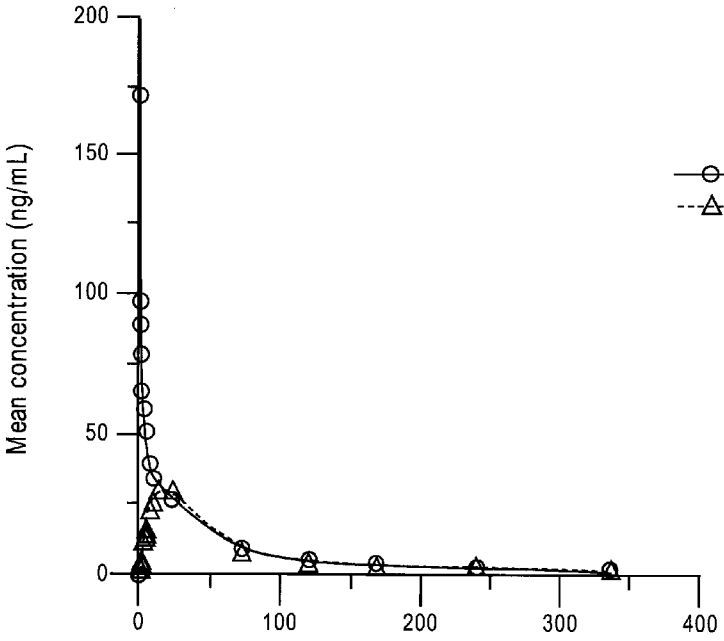

Figure 1(a)

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— *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.

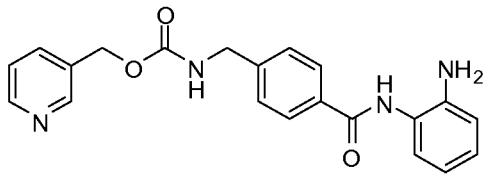
Treatment of Breast Cancer

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.

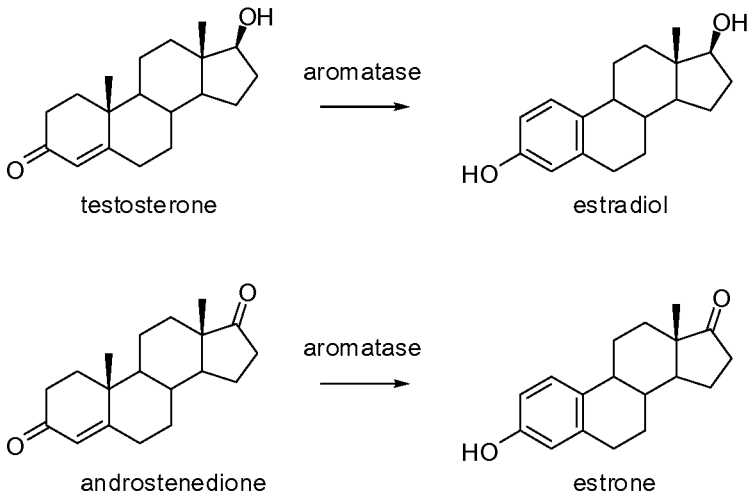
Aromatase is an enzyme of the cytochrome P450 family and a product of the CYP19 gene. The chemical function of aromatase is to convert testosterone to estradiol and androstenedione to estrone.

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.

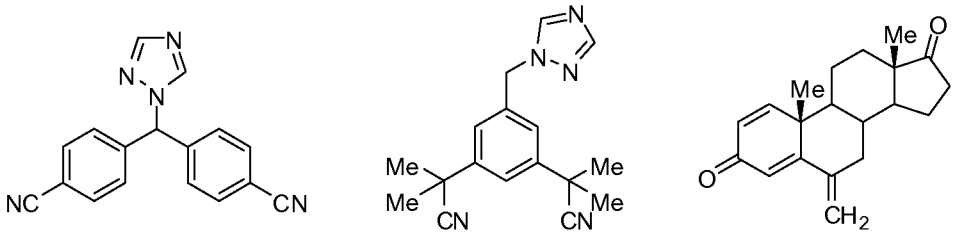


[0043] Subcutaneous fat is a major site of aromatase activity and it has been suggested that plasma estrogen levels correlate with body-mass index (Longcope et al , Metabolism 1986, 35, 235-7). It has been suggested that at menopause, plasma estrogen levels fall from about 110 pg/mL to a much lower level 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 Anastrozole 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 ≥ 9.0 g/dL; platelets ≥ 100.0 x 109/L; ANC ≥ 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.

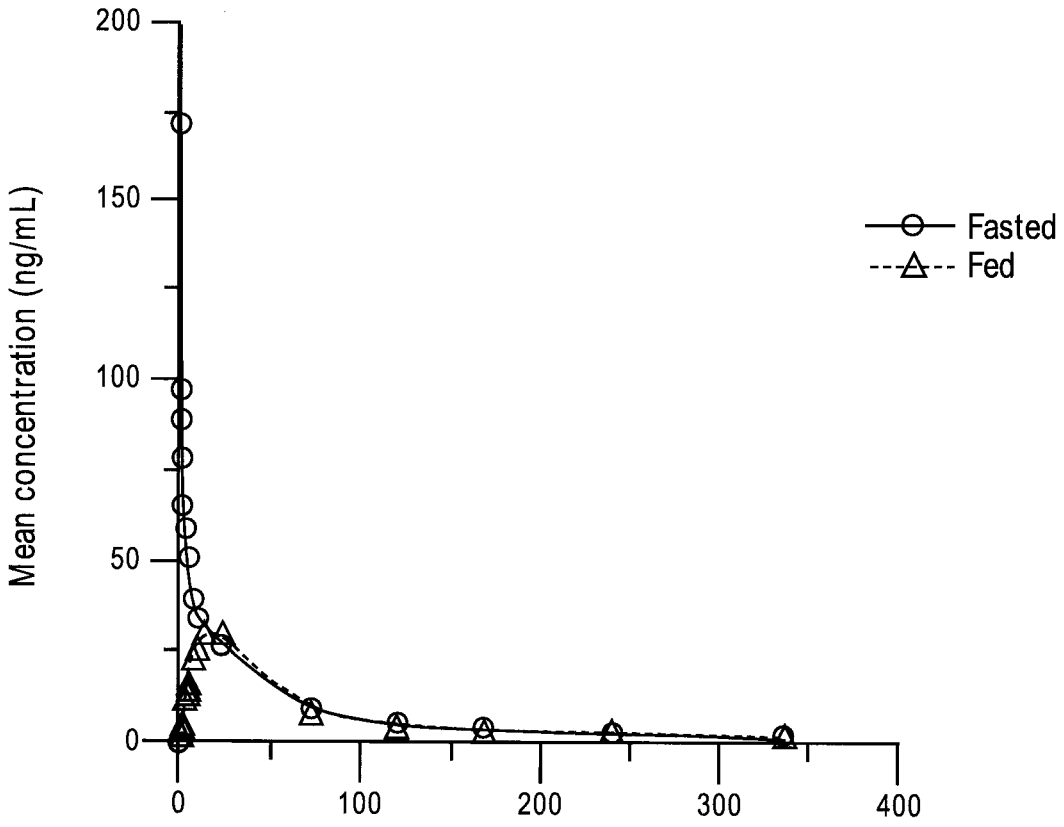


Figure 1(a)

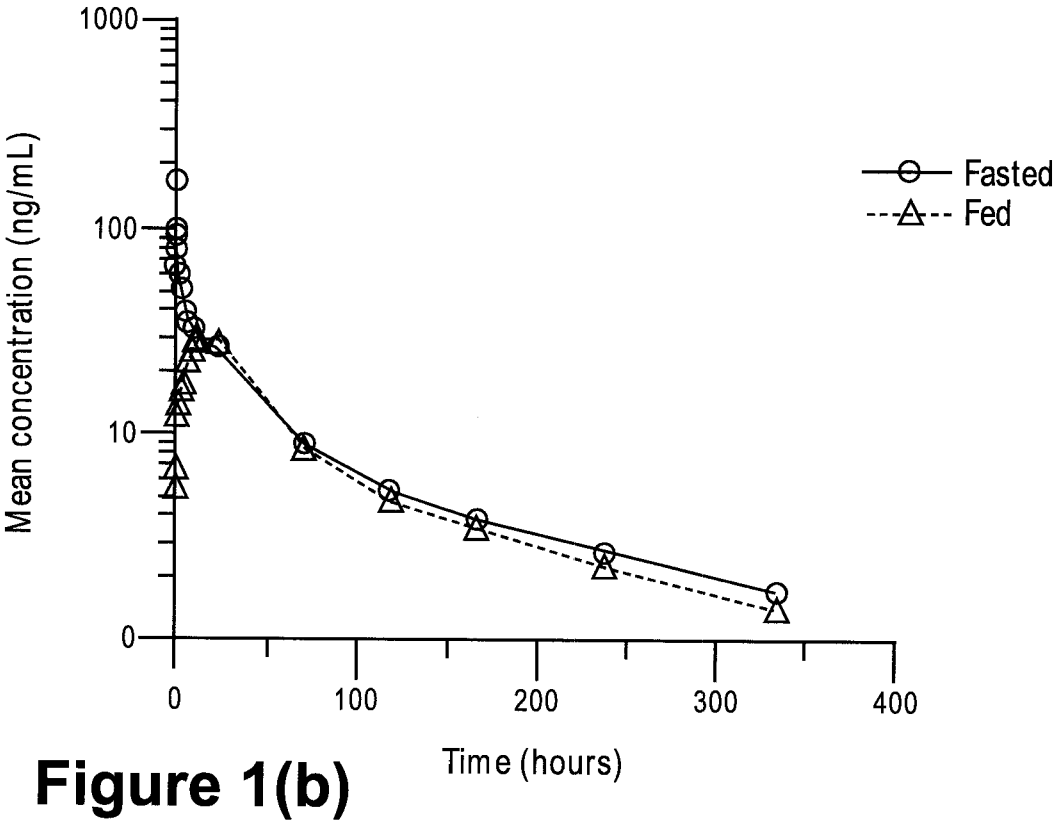


Figure 1(b)

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

Form PCT/ISA/210 (second sheet) (July 2009)

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.

(19)中华人民共和国国家知识产权局

(12)发明专利申请

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代理人 郑霞

(51)Int.Cl.
A61K 31/4406(2006.01)

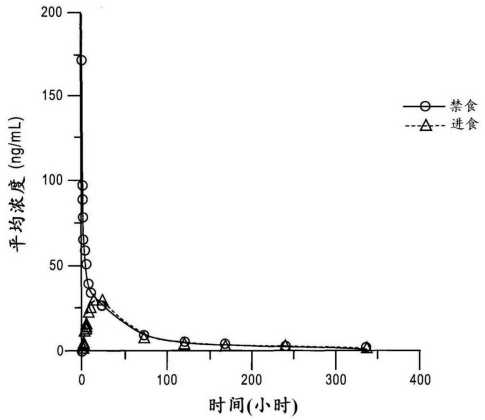
权利要求书5页 说明书35页 附图1页

(54)发明名称

癌症治疗方法

(57)摘要

本文描述了用于治疗受试者的癌症的方法。具体地,提供了用于使用恩替司他和EGFR抑制剂的组合治疗肺癌,或者使用恩替司他和芳香酶抑制剂的组合治疗乳腺癌的方法。进一步地,食物效应对于口服施用恩替司他是明显的。



CN 105492007 A

1.一种治疗有需要的患者的乳腺癌的方法，该方法包括口服施用依西美坦和恩替司他，其中恩替司他施用于禁食患者。

2.根据权利要求1所述的方法，其中施用后恩替司他Tmax少于1小时。

3.根据权利要求1所述的方法，其中施用后恩替司他Tmax少于90分钟。

4.根据权利要求1所述的方法，其中施用后恩替司他Tmax少于2小时。

5.根据权利要求1所述的方法，其中施用后恩替司他Tmax为30分钟至2小时。

6.根据权利要求1-5中任一项所述的方法，其中口服施用恩替司他后，恩替司他Cmax为至少150ng/mL。

7.根据权利要求1-5中任一项所述的方法，其中口服施用恩替司他后，恩替司他Cmax为至少125ng/mL。

8.根据权利要求1-5中任一项所述的方法，其中口服施用恩替司他后，恩替司他Cmax为至少100ng/mL。

9.根据权利要求1-5中任一项所述的方法，其中口服施用恩替司他后，恩替司他Cmax为至少80ng/mL。

10.根据权利要求1-5中任一项所述的方法，其中口服施用恩替司他后，恩替司他Cmax为至少50ng/mL。

11.根据权利要求1-10中任一项所述的方法，其中施用约5mg的恩替司他。

12.根据权利要求1-10中任一项所述的方法，其中施用约10mg的恩替司他。

13.根据权利要求1-10中任一项所述的方法，其中施用约1mg至约20mg的恩替司他。

14.根据权利要求1-13中任一项所述的方法，其中每周一次施用恩替司他。

15.根据权利要求1-14中任一项所述的方法，其中在28-天周期内施用恩替司他。

16.根据权利要求1-15中任一项所述的方法，其中所述患者在施用恩替司他前2小时内不进食。

17.根据权利要求1-15中任一项所述的方法，其中所述患者在施用恩替司他前1小时内不进食。

18.根据权利要求1-17中任一项所述的方法，其中所述患者在施用恩替司他后2小时内不进食。

19.根据权利要求1-17中任一项所述的方法，其中所述患者在施用恩替司他后30分钟内不进食。

20.根据权利要求1-19中任一项所述的方法，其中在与恩替司他施用当日不同的时间施用依西美坦。

21.根据权利要求1-20中任一项所述的方法，其中餐后施用依西美坦。

22.根据权利要求1-20中任一项所述的方法，其中随餐施用依西美坦。

23.根据权利要求1-22中任一项所述的方法，其中每日一次施用约25mg的依西美坦。

24.一种治疗有需要的患者的非小细胞肺癌的方法，该方法包括口服施用厄洛替尼和恩替司他，其中恩替司他施用于禁食患者。

25.根据权利要求24所述的方法，其中施用后恩替司他Tmax少于1小时。

26.根据权利要求24所述的方法，其中施用后恩替司他Tmax少于90分钟。

27.根据权利要求24所述的方法，其中施用后恩替司他Tmax少于2小时。

28.根据权利要求24所述的方法，其中施用后恩替司他Tmax为30分钟至2小时。

29.根据权利要求24-28中任一项所述的方法，其中口服施用恩替司他后，恩替司他Cmax为至少150ng/mL。

30.根据权利要求24-28中任一项所述的方法，其中口服施用恩替司他后，恩替司他Cmax为至少125ng/mL。

31.根据权利要求24-28中任一项所述的方法，其中口服施用恩替司他后，恩替司他Cmax为至少100ng/mL。

32.根据权利要求24-28中任一项所述的方法，其中口服施用恩替司他后恩替司他Cmax为至少80ng/mL。

33.根据权利要求24-28中任一项所述的方法，其中口服施用恩替司他后，恩替司他Cmax为至少50ng/mL。

34.根据权利要求24-33中任一项所述的方法，其中施用约10mg的恩替司他。

35.根据权利要求24-33中任一项所述的方法，其中施用约1mg至约20mg的恩替司他。

36.根据权利要求24-35中任一项所述的方法，其中每14天施用一次恩替司他。

37.根据权利要求24-36中任一项所述的方法，其中施用恩替司他一个月。

38.根据权利要求24-37中任一项所述的方法，其中所述患者在施用恩替司他前2小时内不进食。

39.根据权利要求24-37中任一项所述的方法，其中所述患者在施用恩替司他前1小时内不进食。

40.根据权利要求24-39中任一项所述的方法，其中所述患者在施用恩替司他后1小时内不进食。

41.根据权利要求24-39中任一项所述的方法，其中所述患者在施用恩替司他后30分钟内不进食。

42.根据权利要求24-41中任一项所述的方法，其中在与恩替司他施用当日不同的时间施用厄洛替尼。

43.根据权利要求24-42中任一项所述的方法，其中将厄洛替尼每日一次施用于所述禁食患者。

44.根据权利要求24-43中任一项所述的方法，其中所述患者在施用厄洛替尼前2小时内不进食。

45.根据权利要求24-44中任一项所述的方法，其中所述患者在施用厄洛替尼后1小时内不进食。

46.根据权利要求24-45中任一项所述的方法，其中施用约150mg的厄洛替尼。

47.一种治疗有需要的患者的癌症的方法，该方法包括口服施用恩替司他，其中与在进食情况下施用恩替司他相比，在禁食情况下施用恩替司他导致Cmax增加，并且其中在禁食情况下施用后的Cmax与在进食情况下施用后的Cmax之比为至少约2:1。

48.根据权利要求47所述的方法，其中在禁食情况下施用后的Cmax与在进食情况下施用后的Cmax之比为至少约3:1。

49.根据权利要求47或48所述的方法，其中在禁食情况下施用后的Cmax与在进食情况下施用后的Cmax之比为至少约4:1。

50.根据权利要求47-49中任一项所述的方法，其中在禁食情况下施用后的Cmax与在进食情况下施用后的Cmax之比为至少约5:1。

51.根据权利要求47-50中任一项所述的方法，其中在禁食情况下施用后的Cmax与在进食情况下施用后的Cmax之比为至少约6:1。

52.根据权利要求47-51中任一项所述的方法，其中在禁食情况下施用后的Cmax与在进食情况下施用后的Cmax之比为至少约7:1。

53.根据权利要求47-52中任一项所述的方法，其中所述癌症为肺癌。

54.根据权利要求53所述的方法，其中所述肺癌为非小细胞肺癌。

55.根据权利要求47-52中任一项所述的方法，其中所述癌症为乳腺癌。

56.根据权利要求53或54所述的方法，还包括口服施用EGFR抑制剂。

57.根据权利要求56所述的方法，其中所述EGFR抑制剂为厄洛替尼。

58.根据权利要求57所述的方法，其中在与恩替司他施用当日不同的时间施用厄洛替尼。

59.根据权利要求57或58所述的方法，其中所述患者在施用厄洛替尼前2小时内不进食。

60.根据权利要求57-59中任一项所述的方法，其中所述患者在施用厄洛替尼后1小时内不进食。

61.根据权利要求57-60中任一项所述的方法，其中施用约150mg的厄洛替尼。

62.根据权利要求57-61中任一项所述的方法，其中每日一次施用厄洛替尼。

63.根据权利要求55所述的方法，还包括口服施用芳香酶抑制剂。

64.根据权利要求63所述的方法，其中所述芳香酶抑制剂是依西美坦。

65.根据权利要求64所述的方法，其中在与恩替司他施用当日不同的时间施用依西美坦。

66.根据权利要求64或65所述的方法，其中餐后施用依西美坦。

67.根据权利要求64或65所述的方法，其中随餐施用依西美坦。

68.根据权利要求64-67中任一项所述的方法，其中施用约25mg的依西美坦。

69.根据权利要求64-68中任一项所述的方法，其中每日一次施用依西美坦。

70.根据权利要求47-69中任一项所述的方法，其中所述患者施用约10mg的恩替司他。

71.根据权利要求47-69中任一项所述的方法，其中所述患者施用约5mg的恩替司他。

72.根据权利要求47-69中任一项所述的方法，其中所述患者施用约1mg至约20mg的恩替司他。

73.根据权利要求47-72中任一项所述的方法，其中所述患者在禁食情况下施用恩替司他前2小时内不进食。

74.根据权利要求47-72中任一项所述的方法，其中所述患者在禁食情况下施用恩替司他前1小时内不进食。

75.根据权利要求47-74中任一项所述的方法，其中所述患者在禁食情况下施用恩替司他后2小时内不进食。

76.根据权利要求47-74中任一项所述的方法，其中所述患者在禁食情况下施用恩替司他后30分钟内不进食。

77.根据权利要求47-76中任一项所述的方法，其中所述患者在进食情况下食用高脂肪膳食。

78.一种治疗有需要的患者的癌症的方法，该方法包括口服施用恩替司他，其中与在禁食情况下施用恩替司他相比，在进食情况下施用恩替司他导致Tmax增加，并且其中在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约2:1。

79.根据权利要求78所述的方法，其中在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为约2:1至约5:1。

80.根据权利要求78所述的方法，其中在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为约5:1至约8:1。

81.根据权利要求78所述的方法，其中在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为约8:1至约12:1。

82.根据权利要求78所述的方法，其中在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为约12:1至约15:1。

83.根据权利要求78-82中任一项所述的方法，其中所述癌症为肺癌。

84.根据权利要求83所述的方法，其中所述肺癌为非小细胞肺癌。

85.根据权利要求78-82中任一项所述的方法，其中所述癌症为乳腺癌。

86.根据权利要求83或84所述的方法，还包括口服施用EGFR抑制剂。

87.根据权利要求86所述的方法，其中所述EGFR抑制剂为厄洛替尼。

88.根据权利要求87所述的方法，其中在与恩替司他施用当日不同的时间施用厄洛替尼。

89.根据权利要求87或88所述的方法，其中所述患者在施用厄洛替尼前2小时内不进食。

90.根据权利要求87-89中任一项所述的方法，其中所述患者在施用厄洛替尼后1小时内不进食。

91.根据权利要求87-90中任一项所述的方法，其中施用约150mg的厄洛替尼。

92.根据权利要求87-91中任一项所述的方法，其中每日一次施用厄洛替尼。

93.根据权利要求85所述的方法，还包括口服施用芳香酶抑制剂。

94.根据权利要求93所述的方法，其中所述芳香酶抑制剂是依西美坦。

95.根据权利要求94所述的方法，其中在与恩替司他施用当日不同的时间施用依西美坦。

96.根据权利要求94或95所述的方法，其中餐后施用依西美坦。

97.根据权利要求94或95所述的方法，其中随餐施用依西美坦。

98.根据权利要求94-97中任一项所述的方法，其中施用约25mg的依西美坦。

99.根据权利要求94-98中任一项所述的方法，其中每日一次施用依西美坦。

100.根据权利要求78-99中任一项所述的方法，其中所述患者施用约10mg的恩替司他。

101.根据权利要求78-99中任一项所述的方法，其中所述患者施用约5mg的恩替司他。

102.根据权利要求78-99中任一项所述的方法，其中所述患者施用约1mg至约20mg的恩替司他。

103.根据权利要求78-102中任一项所述的方法，其中所述患者在禁食情况下施用恩替

司他前2小时内不进食。

104.根据权利要求78-102中任一项所述的方法，其中所述患者在禁食情况下施用恩替司他前1小时内不进食。

105.根据权利要求78-104中任一项所述的方法，其中所述患者在禁食情况下施用恩替司他后2小时内不进食。

106.根据权利要求78-104中任一项所述的方法，其中所述患者在禁食情况下施用恩替司他后30分钟内不进食。

107.根据权利要求78-106中任一项所述的方法，其中所述患者在进食情况下食用高脂肪膳食。

108.一种治疗有需要的患者的癌症的方法，该方法包括口服施用恩替司他，其中与在进食情况下施用恩替司他相比，在禁食情况下施用恩替司他时恩替司他的Cmax增加。

109.一种治疗有需要的患者的癌症的方法，该方法包括口服施用恩替司他，其中与在禁食情况下施用恩替司他相比，在进食情况下施用恩替司他时恩替司他的Tmax增加。

癌症治疗方法

交叉引用

[0001] 本申请要求2013年5月3日提交的美国临时申请号61/819,505的权益，该临时申请通过引用全文并入本文。

技术领域

[0002] 本发明涉及基于共施用HDAC抑制剂治疗癌症的方法。

背景技术

[0003] 癌症、肿瘤、肿瘤相关病症以及赘生物疾病状态是严重的并且通常威胁生命的病症。这些疾病和病症(其特征在于快速增殖的细胞生长)仍然是旨在鉴定在其治疗中有效的治疗剂的研究工作的对象。这类药剂可延长患者的生存期、抑制与赘生物相关的快速增殖的细胞生长或实现赘生物的消退。

[0004] 一般说来，手术和放疗是治疗局部限制性癌症时所考虑的首要方式，并提供最佳的预后。一些癌症的化疗通常导致令人失望的生存率但仍可提供生存益处。例如，在肺癌患者中，使用表皮生长因子受体(EGFR)抑制剂化疗方案，如使用厄洛替尼和吉非替尼。如果患者对EGFR抑制剂治疗无反应，则目前所采用的另外的常规治疗提供了有限的益处。在乳腺癌患者中，使用芳香酶抑制剂化疗方案，如使用来曲唑、阿那曲唑或依西美坦。如果患者对芳香酶抑制剂治疗没有反应，则其他常规治疗提供有限的益处。

[0005] 尽管若干EGFR抑制剂已被批准用于治疗肺癌，但EGFR抑制剂治疗受到限制，如由其使用所导致的副作用。令人担心的是，越来越多的观点认为尽管使用EGFR抑制剂治疗肿瘤可在开始时缩小肿瘤的大小，但是肿瘤的大小最终可能变大，这尤其意味着抗药性的形成。厄洛替尼，一种广泛使用的EGFR抑制剂，可代表由于其使用对癌症有效果而用于癌症治疗的治疗剂的类型，但由于其他并不完全清楚的因素，肿瘤产生抗性并进展。

[0006] 尽管若干芳香酶抑制剂已被批准用于治疗早期和晚期乳腺癌，但与大多数治疗剂一样，其使用产生副作用。例如，常见的副作用包括热潮红、血管舒张和恶心。令人担心的是，越来越多的观点认为尽管使用芳香酶抑制剂治疗肿瘤可在开始时缩小肿瘤的大小，但是肿瘤的大小最终可能变大，这尤其意味着抗药性的形成。来曲唑，一种广泛使用的芳香酶抑制剂，可代表用于癌症治疗的治疗剂的类型；它的使用对癌症有影响，但由于其他并不完全清楚的因素，肿瘤产生抗性并进展。

[0007] 组蛋白脱乙酰酶(HDAC)抑制剂为新兴的一类通过染色质重塑和基因表达调节促进血液和实体恶性肿瘤分化和凋亡的治疗剂。已确定了若干HDAC抑制剂，包括苯甲酰胺类(恩替司他(entinostat))、短链脂肪酸类(即苯丁酸钠)；异羟肟酸类(即辛二酰苯胺异羟肟酸(suberoylanilide hydroxamic acid)和曲古抑菌素A(thrichostatin A))；含2-氨基-8-氧代-9,10-环氧-癸酰基部分的环肽(即trapoxin A)和无2-氨基-8-氧代-9,10-环氧-癸酰基部分的环四肽(即FK228)。恩替司他是一种苯甲酰胺HDAC抑制剂，其正在对多种类型的实体瘤和血液癌症进行临床研究。恩替司他可被快速吸收并具有约100小时的半衰期，并

且,重要的是,在施用恩替司他后,组蛋白乙酰化的变化持续数周。

[0008] 因此,需要利用在治疗组合中发现的协同作用治疗癌症的组合物和/或方法,该治疗组合可增加药剂的有效性并减少和/或消除通常与常规治疗相关的副作用。

发明内容

[0009] 一个实施方案提供了一种治疗有需要的患者的癌症的方法,该方法包括口服施用恩替司他,其中与在进食情况下施用恩替司他相比,在禁食情况下施用恩替司他时恩替司他的Cmax增加。

[0010] 一个实施方案提供了一种治疗有需要的患者的癌症的方法,该方法包括口服施用恩替司他,其中与在禁食情况下施用恩替司他相比,在进食情况下施用恩替司他时恩替司他的Tmax增加。

[0011] 一个实施方案提供了一种治疗有需要的患者的乳腺癌的方法,该方法包括口服施用依西美坦和恩替司他,其中恩替司他施用于禁食患者。

[0012] 一个实施方案提供了一种治疗有需要的患者的非小细胞肺癌的方法,该方法包括口服施用厄洛替尼和恩替司他,其中恩替司他施用于禁食患者。

[0013] 一个实施方案提供了一种治疗有需要的患者的癌症的方法,该方法包括口服施用恩替司他,其中与在进食情况下施用恩替司他相比,在禁食情况下施用恩替司他导致Cmax增加,并且其中在禁食情况下施用后的Cmax与在进食情况下施用后的Cmax之比为至少约2:1。

[0014] 一个实施方案提供了一种治疗有需要的患者的癌症的方法,该方法包括口服施用恩替司他,其中与在禁食情况下施用恩替司他相比,在进食情况下施用恩替司他导致Tmax增加,并且其中在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约2:1。

援引并入

[0015] 本说明书中所述的所有出版物、专利和专利申请均通过引用而以相同程度并入本文,犹如特别地且单独地指出每个单独的出版物、专利或专利申请通过引用而并入。专利申请PCT国际专利申请号PCT/US2012/053551、美国专利申请号14/342,354和美国专利申请公开号2013/0150386通过引用并入本文。

附图说明

[0016] 本发明的新颖特征在所附的权利要求书中特别地提出。通过参考以下对利用本发明原理的说明性实施方案加以阐述的详细描述以及附图,将获得对本发明的特征和优点的更好的理解,在这些附图中:

图1提供了在实施例1中描述的食物效应研究的药代动力学分析。

具体实施方式

[0017] 本文提供了基于组蛋白脱乙酰酶(HDAC)抑制剂和芳香酶抑制剂的施用来治疗乳腺癌的方法。该方法包括在无食物的情况下施用HDAC抑制剂。该治疗方法可引入基于在治疗期间所观察到的蛋白质赖氨酸乙酰化水平的患者选择。该方法还可包括治疗,其中HDAC

抑制剂和芳香酶抑制剂的施用补充以一种或多种治疗剂或疗法。

[0018] 本文提供了基于HDAC抑制剂和表皮生长因子受体(EGFR)抑制剂的施用来治疗肺癌的方法。该方法包括在无食物的情况下施用HDAC抑制剂。该方法还可包括治疗，其中HDAC抑制剂和EGFR抑制剂的施用补充以一种或多种治疗剂或疗法。

[0019] 一个实施方案提供了治疗有需要的患者的癌症的方法，该方法包括口服施用恩替司他，其中与在进食情况下施用恩替司他相比，在禁食情况下施用恩替司他导致Cmax增加，并且其中在禁食情况下施用后的Cmax与在进食情况下施用后的Cmax之比为至少约2:1。另一个实施方案提供了治疗癌症的方法，其中在禁食情况下施用后的Cmax与在进食情况下施用后的Cmax之比为至少约3:1。另一个实施方案提供了治疗癌症的方法，其中在禁食情况下施用后的Cmax与在进食情况下施用后的Cmax之比为至少约4:1。另一个实施方案提供了治疗癌症的方法，其中在禁食情况下施用后的Cmax与在进食情况下施用后的Cmax之比为至少约5:1。另一个实施方案提供了治疗癌症的方法，其中在禁食情况下施用后的Cmax与在进食情况下施用后的Cmax之比为至少约6:1。另一个实施方案提供了治疗癌症的方法，其中在禁食情况下施用后的Cmax与在进食情况下施用后的Cmax之比为至少约7:1。另一个实施方案提供了治疗癌症的方法，其中所述癌症为肺癌。另一个实施方案提供了治疗癌症的方法，其中所述肺癌为非小细胞肺癌。另一个实施方案提供了治疗癌症的方法，其中所述癌症为乳腺癌。另一个实施方案提供了治疗癌症的方法，该方法还包括口服施用EGFR抑制剂。另一个实施方案提供了治疗癌症的方法，其中所述EGFR抑制剂为厄洛替尼。另一个实施方案提供了治疗癌症的方法，其中在与恩替司他施用当日不同的时间施用厄洛替尼。另一个实施方案提供了治疗癌症的方法，其中所述患者在施用厄洛替尼前2小时内不进食。另一个实施方案提供了治疗癌症的方法，其中所述患者在施用厄洛替尼后1小时内不进食。另一个实施方案提供了治疗癌症的方法，其中施用约150mg的厄洛替尼。另一个实施方案提供了治疗癌症的方法，其中每日一次施用厄洛替尼。另一个实施方案提供了治疗癌症的方法，该方法还包括口服施用芳香酶抑制剂。另一个实施方案提供了治疗癌症的方法，其中所述芳香酶抑制剂是依西美坦。另一个实施方案提供了治疗癌症的方法，其中在与恩替司他施用当日不同的时间施用依西美坦。另一个实施方案提供了治疗癌症的方法，其中餐后施用依西美坦。另一个实施方案提供了治疗癌症的方法，其中随餐施用依西美坦。另一个实施方案提供了治疗癌症的方法，其中施用约25mg的依西美坦。另一个实施方案提供了治疗癌症的方法，其中每日一次施用依西美坦。另一个实施方案提供了治疗癌症的方法，其中所述患者施用约10mg的恩替司他。另一个实施方案提供了治疗癌症的方法，其中所述患者施用约5mg的恩替司他。另一个实施方案提供了治疗癌症的方法，其中所述患者施用约1mg至约20mg的恩替司他。另一个实施方案提供了治疗癌症的方法，其中所述患者在禁食情况下施用恩替司他前2小时内不进食。

另一个实施方案提供了治疗癌症的方法，其中所述患者在禁食情况下施用恩替司他前1小时内不进食。另一个实施方案提供了治疗癌症的方法，其中所述患者在禁食情况下施用恩替司他后2小时内不进食。另一个实施方案提供了治疗癌症的方法，其中所述患者在禁食情况下施用恩替司他后30分钟内不进食。另一个实施方案提供了治疗癌症的方法，其中所述患者在进食情况下食用高脂肪膳食。

[0020] 一个实施方案提供了治疗有需要的患者的癌症的方法，该方法包括口服施用恩替

司他，其中与在禁食情况下施用恩替司他相比，在进食情况下施用恩替司他导致Tmax增加，并且其中在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约2:1。另一个实施方案提供了治疗癌症的方法，其中在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为约2:1至约5:1。另一个实施方案提供了治疗癌症的方法，其中在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为约5:1至约8:1。另一个实施方案提供了治疗癌症的方法，其中在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为约8:1至约12:1。另一个实施方案提供了治疗癌症的方法，其中在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为约12:1至约15:1。另一个实施方案提供了治疗癌症的方法，其中所述癌症为肺癌。另一个实施方案提供了治疗癌症的方法，其中所述肺癌为非小细胞肺癌。另一个实施方案提供了治疗癌症的方法，其中所述癌症为乳腺癌。另一个实施方案提供了治疗癌症的方法，该方法还包括口服施用EGFR抑制剂。另一个实施方案提供了治疗癌症的方法，其中所述EGFR抑制剂为厄洛替尼。另一个实施方案提供了治疗癌症的方法，其中在与恩替司他施用当日不同的时间施用厄洛替尼。另一个实施方案提供了治疗癌症的方法，其中所述患者在施用厄洛替尼前2小时内不进食。另一个实施方案提供了治疗癌症的方法，其中所述患者在施用厄洛替尼后1小时内不进食。另一个实施方案提供了治疗癌症的方法，其中施用约150mg的厄洛替尼。另一个实施方案提供了治疗癌症的方法，其中每日一次施用厄洛替尼。另一个实施方案提供了治疗癌症的方法，该方法还包括口服施用芳香酶抑制剂。另一个实施方案提供了治疗癌症的方法，其中所述芳香酶抑制剂是依西美坦。另一个实施方案提供了治疗癌症的方法，其中在与恩替司他施用当日不同的时间施用依西美坦。另一个实施方案提供了治疗癌症的方法，其中餐后施用依西美坦。另一个实施方案提供了治疗癌症的方法，其中随餐施用依西美坦。另一个实施方案提供了治疗癌症的方法，其中施用约25mg的依西美坦。另一个实施方案提供了治疗癌症的方法，其中每日一次施用依西美坦。另一个实施方案提供了治疗癌症的方法，其中所述患者施用约10mg的恩替司他。另一个实施方案提供了治疗癌症的方法，其中所述患者施用约5mg的恩替司他。另一个实施方案提供了治疗癌症的方法，其中所述患者施用约1mg至约20mg的恩替司他。另一个实施方案提供了治疗癌症的方法，其中所述患者在禁食情况下施用恩替司他前2小时内不进食。另一个实施方案提供了治疗癌症的方法，其中所述患者在禁食情况下施用恩替司他前1小时内不进食。另一个实施方案提供了治疗癌症的方法，其中所述患者在禁食情况下施用恩替司他后2小时内不进食。另一个实施方案提供了治疗癌症的方法，其中所述患者在禁食情况下施用恩替司他后30分钟内不进食。另一个实施方案提供了治疗癌症的方法，其中所述患者在进食情况下食用高脂肪膳食。

[0021] 为便于理解本文所述的公开内容，很多术语定义如下。

[0022] 如本文所用，“异常的细胞生长”是指不依赖于正常调节机制(例如，丧失接触抑制)的细胞生长，包括正常细胞的异常生长和异常细胞的生长。

[0023] 如本文所述的“瘤形成”是异常的、未调节的和紊乱的细胞增殖，其与正常细胞的区别在于自发生长和体细胞突变。随着赘生细胞生长和分裂，它们将它们的基因突变和增殖特性传递给后代细胞。赘生物或肿瘤是赘生细胞的积累。在一些实施方案中，所述赘生物可以是良性的或恶性的。

[0024] 如本文所用，“转移”是指肿瘤细胞通过淋巴管或血管扩散。转移还指肿瘤细胞通

过直接蔓延迁移穿过浆膜腔或蛛网膜下空间或其他空间。通过转移过程，肿瘤细胞向身体的其他区域的迁移在远离初始出现部位的区域形成肿瘤。

[0025] 如本文所讨论的，“血管发生”在肿瘤形成和转移中是显著的。已发现血管生成因子与若干实体瘤如横纹肌肉瘤、视网膜母细胞瘤、尤因肉瘤、神经母细胞瘤和骨肉瘤相关。在无血液供应来提供养分并除去细胞废物的情况下，肿瘤无法扩张。血管发生至关重要的肿瘤包括实体瘤，如肾细胞癌、肝细胞癌，和良性肿瘤如听神经瘤和神经纤维瘤。血管发生与血液肿瘤如白血病相关。据信血管发生在引起白血病的骨髓异常中起作用。阻止血管发生可使癌性肿瘤的生长和由于肿瘤的存在而导致的对受试者的损伤停止。

[0026] 术语“受试者”是指动物，包括但不限于灵长类(例如，人)、牛、绵羊、山羊、马、狗、猫、兔、大鼠或小鼠。术语“受试者”和“患者”在本文提及例如哺乳动物受试者如人受试者时可互换使用。

[0027] 术语“治疗”意在包括减轻或消除病症、疾病或病状；或一种或多种与所述病症、疾病或病状相关的症状；或减轻或根除所述病症、疾病或病状本身的病因。

[0028] 术语“治疗有效量”是指当施用时，足以预防所治疗的病症、疾病或病状的一种或多种症状的发展或减轻一定程度的化合物的量。术语“治疗有效量”还指足以引发研究者、兽医、医生或临床医师所寻找的细胞、组织、系统、动物或人的生物学或医学应答的化合物的量。

[0029] 术语“药学可接受的载体”、“药学可接受的赋形剂”、“生理学可接受的载体”或“生理学可接受的赋形剂”是指药学可接受的材料、组合物或媒介物，如液体或固体填充剂、稀释剂、赋形剂、溶剂或包封材料。每种组分从与药物制剂的其他成分相容的意义上来说必须是“药学上可接受的”。它还必须适用于与人和动物的组织或器官接触而无过度的毒性、刺激、变应性应答、免疫原性或其他问题或并发症，并与合理的受益/风险比相称。参见Remington:The Science and Practice of Pharmacy,第21版;Lippincott Williams& Wilkins:Philadelphia,PA,2005;Handbook of Pharmaceutical Excipients,第5版;Rowe等人,Eds.,The Pharmaceutical Press and the American Pharmaceutical Association:2005;和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] 术语“药物组合物”是指本文所公开的化合物与其他化学组分如稀释剂或载体的混合物。所述药物组合物促进了向生物体施用所述化合物。本领域中存在的多种施用化合物的技术包括但不限于口服、注射、气雾剂、胃肠外和局部施用。药物组合物还可通过将化合物与无机酸或有机酸如盐酸、氢溴酸、硫酸、硝酸、磷酸、甲磺酸、乙磺酸、对甲苯磺酸、水杨酸等反应而获得。

[0031] 术语“禁食”、“禁食的”或“无食物”被定义为通常意指从本文所述的治疗剂施用之前至少约30分钟到本文所述的治疗剂施用之后至少约30分钟之间的时间段期间没有进食的情况。在一些情况下，从本文所述的治疗剂施用之前至少约2小时到本文所述的治疗剂施用之后至少约1小时没有进食。在一些情况下，从本文所述的治疗剂施用之前至少约1小时到本文所述的治疗剂施用之后至少约1小时没有进食。在一些情况下，从本文所述的治疗剂施用之前至少约1小时到本文所述的治疗剂施用之后至少约2小时没有进食。

[0032] 术语“进食情况”是指已经用过膳食的情况。在一些情况下，该食物是高脂肪或高热量膳食。高热量膳食可包括但不限于，包含500卡路里或更多卡路里、约300到约800卡路里、约500卡路里到约1,000卡路里以及约800卡路里到约1,500卡路里的膳食。在一些情况下，高脂肪膳食包括但不限于，来自脂肪的热量占每日热量摄入的百分比为约20％到约50％、约30％到约60％以及约40％到约70％的膳食。在一些实施方案中，该膳食不是高脂肪的。在一些实施方案中，该膳食不是高热量的。

[0033] 术语“生物利用度”通常指从治疗剂吸收并变得在作用部位可利用的活性成分的比率和程度。对于口服剂型，生物利用度与活性成分从口服剂型中释放并移动至作用部位的过程相关。在量上，术语“口服生物利用度”或“％F”被定义为AUC_{oral}/AUC_{iv}，其中AUC_{oral}是口服施用后测得的AUC，而AUC_{iv}是静脉内施用后测得的AUC。

[0034] “AUC”是指药物-浓度曲线下面积。“AUC^{0-t}”是指从零至时间t的药物-浓度曲线下面积。“AUClast”是指从零到药物-浓度曲线的最后数据点的药物-浓度曲线下面积。“$AUC^{0-\infty}$”或“AUCinf”是指从零到无限时间的药物-浓度曲线下面积。

[0035] “$t_{1/2}$”是指所指物种的消除半衰期。“t_{max}”是指所指物种的最大浓度的时间。“C_{max}”是指所指物种的最大浓度。

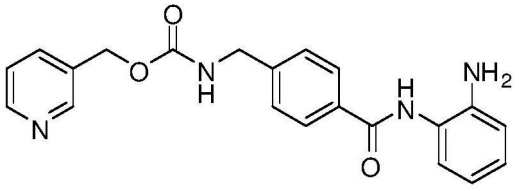
乳腺癌的治疗

组蛋白脱乙酰基酶

[0036] HDAC是包括至少18种酶的家族，其分为3类(第I、II和III类)。第I类HDAC包括但不限于HDAC 1、2、3和8。第I类HDAC可见于细胞核中并且据认为与转录控制抑制物有关。第II类HDAC包括但不限于HDAC 4、5、6、7和9，并且可见于细胞质和细胞核中。据信第III类HDAC是NAD依赖性蛋白质，且包括但不限于Sirtuin家族蛋白质的成员。Sirtuin蛋白质的非限制性实例包括SIRT1-7。如本文所用，术语“选择性HDAC抑制剂”是指不与全部3类HDAC相互作用的HDAC抑制剂。

HDAC抑制剂

[0037] HDAC抑制剂可被广义地分为泛HDAC抑制剂和选择性HDAC抑制剂。尽管已知的HDAC抑制剂存在巨大的结构多样性，但它们共享共同的特征：与酶活性位点相互作用的部分和位于通往该活性位点的通道内部的侧链。这点可参见异羟肟酸类如SAHA，其中据信异羟肟酸基与所述活性位点相互作用。在缩肽类的情况下，据信细胞内二硫键的还原产生了连接至4-碳烯基链的游离巯基(其与所述活性位点相互作用)。HDAC抑制剂之间的区别在于它们与HDAC通道的边缘(其位于通往该活性位点的通道的相反端)相互作用的方式。据信，这种HDAC抑制剂和通道边缘之间的相互作用至少部分地解释了在泛HDAC抑制剂如SAHA和选择性HDAC抑制剂如缩肽类之间所观测到的HDAC选择性的一些差异。特别优选的HDAC抑制剂为恩替司他。恩替司他的化学名为N-(2-氨基苯基)-4-[N-(吡啶-3-基)甲氧基羰基氨基-甲基]-苯甲酰胺，并且其化学结构如下所示。



恩替司他的化学结构

芳香酶

[0038] 雌激素是一种雌性性激素并且在机体中具有很多功能。已发现约80％的乳腺癌肿瘤过度表达雌激素受体并对雌激素的存在积极应答。在绝经后的女性中，卵巢雌激素产生降低并且血浆雌激素水平通常低于绝经前的女性。

[0039] 绝经后女性雌激素的残余来源是从雄激素合成雌激素，这由芳香酶催化。芳香酶活性的抑制将导致雌激素水平降低，从而降低对雌激素的存在积极应答的乳腺癌肿瘤的生长。

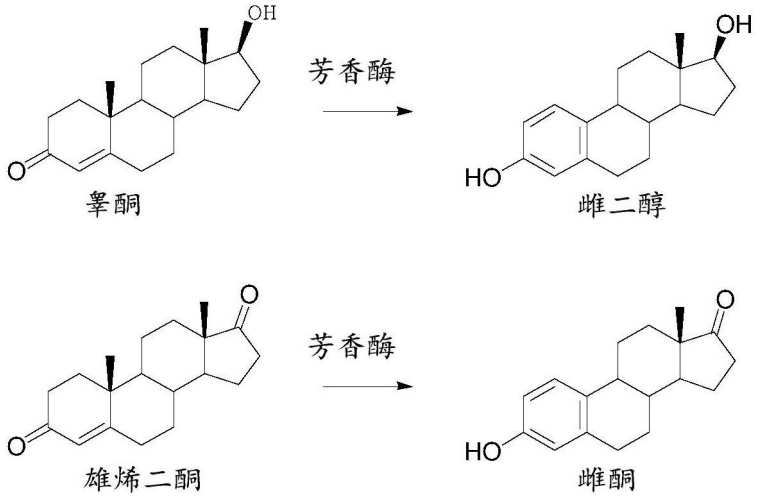
芳香酶是细胞色素P450家族的酶，且为CYP19基因的产物。芳香酶的化学功能是将睾酮转化成雌二醇和将雄烯二酮转化成雌酮。

芳香酶抑制剂

[0040] 芳香酶抑制剂通过阻断芳香酶将雄激素转化成雌激素而降低机体的雌激素。对于早期乳腺癌的治疗，可使用一些芳香酶抑制剂作为辅助疗法代替他莫昔芬或在使用他莫昔芬2年或2年以上后使用芳香酶抑制剂。对于转移性乳腺癌的治疗，正在临床试验中测试芳香酶抑制剂以将其与他莫昔芬的激素疗法比较。

[0041] 如本文所述，“芳香酶抑制剂”为抑制芳香酶的活性的分子。本领域技术人员使用诸如标准药理学测试程序(其测量1,2-^{3}H-雄烯二酮向雌酮转化的抑制)等方法可容易地鉴别作为芳香酶抑制剂的化合物。

[0042] 简言之，通过Thompson和Siiteri(J.Biol.Chem.,Vol.249,p.5364(1974))所述的方法从人胎盘制备微粒体部分。将这样获得的微粒体制剂冻干并在-40℃保存。将所述人胎盘微粒体加入至1,2-^{3}H-雄烯二酮并在37℃孵育20分钟。通过$^{3}H_2O$进入孵育培养基的损失检测该标记底物的芳香化的量。通过氯仿萃取移出底物，随后吸附至悬浮液中的炭。通过离心法除去炭并在液体闪烁计数器中对无甾体培养基进行计数。通过在加入微粒体之前将组合物加入该孵育培养基中测试该组合物的芳香酶抑制活性。在有或无该组合物的条件下获得的相对cpm用于计算雄烯二酮芳香化成雌酮的抑制百分比。可根据雄烯二酮至雌酮的芳香化降低至对照值的50％时的测试化合物的浓度以图形方式测定IC_{50}值。



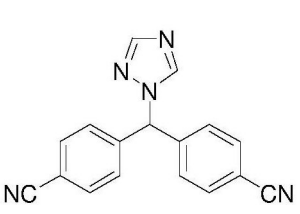
[0043] 皮下脂肪是主要的芳香酶活性位点，且已经表明血浆雌激素水平与体重指数相关(Longcope等人,Metabolism 1986,35,235-7)。已经表明在绝经期，血浆雌激素水平从约110pg/mL降至低很多的水平，约7pg/mL。然而，已发现，在绝经期后的女性中，瘤内雌二醇的

浓度约为血浆中雌二醇浓度的10倍，这可能是由于肿瘤内的芳香酶活性所致。

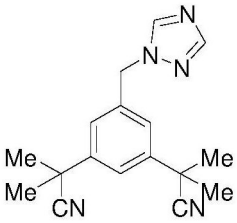
[0044] 芳香酶的抑制作为乳腺癌的治疗选择已得到研究并获得一些成功。目前三类芳香酶抑制剂被批准在美国上市用于治疗绝经后女性中各个阶段的乳腺癌。来曲唑(Femara®)适用于若干治疗选择，包括之前经他莫昔芬治疗5年的绝经后女性的早期乳腺癌的延伸辅助治疗、激素受体阳性(或未知)的绝经后女性局部晚期或转移性乳腺癌的治疗，和抗雌激素疗法后疾病进展的绝经后女性的晚期乳腺癌治疗。

[0045] 阿那曲唑(Arimidex®)适用于若干治疗选择，包括激素受体(+)的绝经后女性早期乳腺癌的辅助治疗、激素受体(+)(或未知)的绝经后女性局部晚期或转移性乳腺癌和他莫昔芬疗法后疾病进展的绝经后女性的晚期乳腺癌的一线治疗。

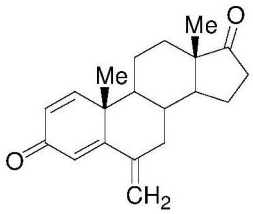
[0046] 依西美坦(阿诺新®(Aromasin®))适用于若干治疗选择，包括已接受他莫昔芬治疗2-3年的雌激素受体(+)的绝经后女性早期乳腺癌和他莫昔芬疗法后疾病进展的绝经后女性晚期乳腺癌的辅助治疗。



来曲唑



阿那曲唑



依西美坦

这些药物可分为两类：(第1类)依西美坦基于甾体化学结构，(第2类)来曲唑和阿那曲唑基于非甾体化学结构。临床试验表明在晚期ER(+)疾病的治疗中来曲唑优于他莫昔芬。在早期疾病中，在降低复发风险方面，使用阿那曲唑辅助疗法似乎优于他莫昔芬疗法。近期的临床试验结果使得芳香酶抑制剂代替他莫昔芬成为乳腺癌治疗的医护标准。

乳腺癌

[0047] 今天，在美国的女性中，乳腺癌仍是最常见的经诊断的癌症。1/8的美国女性有发展成乳腺癌的危险。年龄、家族史、饮食和遗传因素均被确定为乳腺癌的危险因素。乳腺癌是女性的第二大死因。

HER2/neu阳性乳腺癌

[0048] 与HER2/neu过度表达相关的癌症包括乳腺癌、卵巢癌、子宫内膜癌、前列腺癌、胃癌、唾液腺癌、胰腺癌、结直肠癌、口腔癌和非小细胞肺癌。乳腺癌已成为抗HER2/neu治疗的焦点。

[0049] 大约25-30％的乳腺癌具有HER2/neu基因扩增或其蛋白质产物的过度表达。乳腺癌中该受体的过度表达与增加的疾病复发和较差的预后相关。

激素阳性癌症

[0050] 许多乳腺癌需要雌激素来生长。在绝经女性中，雌激素的主要来源是通过雄激素转化成雌激素。如上所述，该过程通过芳香酶进行。

三阴性乳腺癌

[0051] 在三阴性乳腺癌(其中该癌症为雌激素受体阴性、孕酮受体阴性和HER2阴性)的治疗中，本文所述的组合物和疗法可与其他治疗剂联合。仅举例来说，这类药剂包括西妥昔单

抗、紫杉醇、多西紫杉醇、紫杉烷制剂，例如Abraxane®(ABI-007)、紫杉醇-聚氧乙烯蓖麻油、聚谷紫杉醇和紫杉醇注射乳剂(PIE)。当与HER2过度表达相关的癌症存在但由于在量化HER2表达时采用的试验中的技术限制未检测到时，这些组合可能是有利的。

[0052] 激素疗法是雌激素受体阳性(ER+)乳腺癌(BC)的主要治疗方法。由于激素药剂的临床活性和总体有利的副作用谱和耐受性，医护标准通常包括依序使用激素药剂直至抗性的发展和/或内脏危象要求转变成化疗。在绝经后女性中，芳香酶抑制剂(AI)是一类优选的抗雌激素疗法，其通过阻断内源性雌激素合成起作用。依西美坦是一种可逆性地与芳香酶结合并使其失活的甾体芳香酶抑制剂，其效力已在经非甾体芳香酶抑制剂，NSAI；即来曲唑或阿那曲唑治疗后进展的转移性情况中得到证明(Chia S,Gradishar W,Mauriac L等人: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] 晚期乳腺癌中对激素疗法的抗性的形成是一个巨大的挑战。推定的抗性机制包括雌激素非依赖性生长、对低雌激素浓度的超敏反应、细胞周期蛋白D1过度表达、组成型核因子κB(NFκB)激活、生长因子信号途径的上调和雌激素受体α(ERα)表达的下调。这些途径和机制为治疗性干预提供了潜在的靶点。恩替司他是一种新型的、口服的组蛋白脱乙酰基酶(HDAC)抑制剂，其对第1类HDAC具有高特异性并具有独特的药理学谱，允许每周给药。HDAC抑制导致肿瘤和外周血细胞中蛋白质赖氨酸乙酰化水平升高，其可作为活性的潜在药效学标记物的替代物。恩替司他对第1类HDAC的特异性使其区别于美国(US)食品药品监督管理局(FDA)批准的HDAC抑制剂(HDACi)vorinstat(Zolinza®)和罗米地新(romidepsin)(Istodax®)。在临床前，已证明恩替司他可抑制ERα阳性肿瘤生长并由于雌激素非依赖性生长因子信号途径的下调而恢复激素敏感性，使ERα水平正常化并增加芳香酶水平。(Sabnis GJ,Goloubeva O,Chumsri S等人:Functional activation of the estrogen receptor-αand 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-7CaXenografts to AIs through Modulation of Her 2.Presented at the 33rd Annual San Antonio Breast Cancer Symposium,San Antonio,TX,December 8-12,2010)。本文所述的具体临床试验结果表明，在ER+乳腺癌中联合使用恩替司他和依西美坦可抑制激素疗法抗性机制，从而使细胞对使用依西美坦的抗雌激素疗法敏感。

其他疗法

[0054] 可被有利地采用与本文所公开的疗法组合的可用的其他乳腺癌治疗方法包括但不限于，放疗、化疗、抗体疗法和酪氨酸激酶抑制剂作为辅助疗法。

[0055] 放疗是一种使用高能X射线或其他类型的放射线杀死癌细胞或阻止其生长的癌症治疗方法。化疗是一种使用药物通过杀死细胞或停止其分裂而停止癌细胞生长的癌症治疗方法。当口服化疗剂或将其注射到静脉或肌肉内时，药物进入血流并可达到遍布全身的癌

症细胞(全身化疗)。当直接将化疗剂放置于脊柱、器官或体腔如腹部中时,药物主要影响这些区域中的癌细胞(局部化疗)。给予化疗的方法取决于所治疗癌症的类型和阶段。

[0056] 不同的用于治疗乳腺癌的化疗剂是本领域已知的。用于治疗乳腺癌的细胞毒性剂包括多柔比星、环磷酰胺、甲氨蝶呤、5-氟尿嘧啶、丝裂霉素C、米托蒽醌、紫杉醇、紫杉烷制剂,仅举例来说,如Abraxane®(ABI-007)、紫杉醇-聚氧乙烯蓖麻油、聚谷紫杉醇和紫杉醇注射乳剂(PIE)、吉西他滨、多西紫杉醇、卡培他滨和表柔比星。

[0057] 其他针对乳腺癌的化疗包括使用一种或多种以下药剂的治疗:苯达莫司汀、卡铂(例如,Paraplatin®)、卡莫司汀(例如,BCNU®)、苯丁酸氮芥(例如,Leukeran®)、顺铂(例如,Platinol®)、环磷酰胺注射液(例如,Cytoxan®)、口服环磷酰胺(例如,Cytoxan®)、达卡巴嗪(例如,DTIC®)、异环磷酰胺(例如,ifex®)、洛莫司汀(例如,CCNU®)、双氯乙基甲胺(mechlorethamine)(例如,氮芥、Mustargen®)、美法仑(例如,Alkeran®)、丙卡巴肼(例如,Matulane®)、博来霉素(例如,Blenoxane®)、多柔比星(例如,Adriamycin®、Rubex®)、表柔比星、伊达比星(例如,Idamycin®)、米托蒽醌(例如,Novantrone®)、吉西他滨(例如,Gemzar®)、口服巯嘌呤(例如,Purinethol®)、甲氨蝶呤、喷司他丁IV(例如,Nipent®)、口服硫鸟嘌呤(例如,Lanvis®)、口服依托泊苷(例如,VP-16、VePesid®、Etopophos)、依托泊苷IV(例如,VP-16、VePesid®、Etopophos)、长春碱(例如,Velban®)、长春新碱(例如,Oncovin®)、长春瑞滨(例如,Navelbine®)、地塞米松(例如,Decadron®)、甲泼尼龙(例如,Medrol®)和泼尼松(例如,Deltasone®)。

[0058] 单克隆抗体疗法是一种使用在实验室中从单一类型的免疫系统细胞制得的抗体的癌症治疗。这些抗体可识别癌细胞上的物质或可帮助癌细胞生长的正常物质。所述抗体附着于这些物质上并杀死癌细胞,阻断其生长或阻碍其扩散。单克隆抗体通过输注给予。它们可单独使用或用于直接携带药物、毒素或放射性物质至癌细胞。单克隆抗体也可与作为辅助疗法的化疗联合使用。

[0059] 曲妥珠单抗(Herceptin®)是一种阻断生长因子蛋白质HER2(其将生长信号传送至乳腺癌细胞)作用的单克隆抗体。

[0060] 曲妥珠单抗可作为单一药剂引起临床应答并且当加入针对晚期HER2阳性乳腺癌的化疗时可改善生存期。然而,一些患者对曲妥珠单抗并不应答,而且大多数最终形成临床抗性。对天然的和获得的曲妥珠单抗抗性机制知之甚少。已报道了一项利用基于细胞系的方法描述与抗性相关的遗传和蛋白质改变的研究(D.Tripathy等人Journal of Clinical Oncology,2005 Vol 23,No 16S,3121)。这些研究者研究了两种HER2阳性乳腺癌细胞系(BT474和SKBR3),将其在曲妥珠单抗的存在下连续传代直至记录到体外抗性。抗性细胞系出现在12个月后,并且在无曲妥珠单抗的条件下展现出3倍更快的生长速率。在曲妥珠单抗暴露后,相对于抗性细胞在敏感细胞中观测到G0/G1停滞(84%相对68%),较少细胞在S期(3%相对14%)。与敏感细胞相比,抗性细胞系在使用曲妥珠单抗的基因表达、趋化因子受

体CXCR4和有丝分裂检查点调节剂的上调以及PTEN的下调中表现出较少变化。

[0061] 可有利地与本文所公开的组合物和疗法联合的其他说明性治疗可包括但不限于施用以下药剂，其包括但不限于拉帕替尼，其单独使用或与卡培他滨、多西紫杉醇、表柔比星、埃博霉素A、B或D、醋酸戈舍瑞林、紫杉醇、帕米膦酸盐、贝伐珠单抗或曲妥珠单抗联合使用。

[0062] 在一些实施方案中，所述其他治疗包括化疗，该化疗包括向受试者施用多柔比星、环磷酰胺、紫杉醇、拉帕替尼、卡培他滨、曲妥珠单抗、贝伐珠单抗、吉西他滨、艾瑞布林或白蛋白结合型紫杉醇(nab-paclitaxel)中的一种或多种。

用于治疗乳腺癌的方法

[0063] 一个实施方案提供了治疗患者的乳腺癌的方法，其包括：(i)在施用恩替司他-芳香酶抑制剂联合治疗之前测定蛋白质赖氨酸乙酰化水平，(ii)施用恩替司他-芳香酶抑制剂联合治疗，(iii)在施用恩替司他-芳香酶抑制剂联合治疗之后测定蛋白质赖氨酸乙酰化水平，(iv)将恩替司他-芳香酶抑制剂联合治疗施用之后的蛋白质赖氨酸乙酰化水平与恩替司他-芳香酶抑制剂联合治疗施用之前的蛋白质赖氨酸乙酰化水平相比较，以及(v)如果恩替司他-芳香酶抑制剂联合治疗施用之后的蛋白质赖氨酸乙酰化水平高于恩替司他-芳香酶抑制剂联合治疗施用之前的蛋白质赖氨酸乙酰化水平，则继续采用恩替司他-芳香酶抑制剂联合治疗的治疗。在一些情况下，向禁食患者施用恩替司他。

[0064] 一个实施方案提供了治疗患者的乳腺癌的方法，其包括：(i)施用恩替司他-芳香酶抑制剂联合治疗，以及(ii)确定所述治疗过程中蛋白质赖氨酸乙酰化水平相比于治疗前蛋白质赖氨酸乙酰化水平的变化。在一些情况下，向禁食患者施用恩替司他。

[0065] 一个实施方案提供了治疗患者的乳腺癌的方法，其包括：(i)确定施用前蛋白质赖氨酸乙酰化的水平，(ii)施用恩替司他-芳香酶抑制剂联合治疗，以及(iii)确定治疗过程中的蛋白质赖氨酸乙酰化水平。在一些情况下，向禁食患者施用恩替司他。

[0066] 期望提高治疗剂如恩替司他的口服生物利用度，从而增加对患者的治疗效果程度。通常，食物对治疗剂的生物利用度具有可变的影响。治疗剂与食物之间的相互作用可导致减少的、延迟的或增加的全身药物可用性。食物可在以下阶段与治疗剂相互作用：(i)胃肠吸收之前和胃肠吸收期间；(ii)分布期间；(iii)代谢期间；以及(iv)排泄期间。在一个实施方案中，当与食物一起施用时，恩替司他的生物利用度降低。

[0067] 食物可通过延迟胃排空和延长肠道通过时间来影响暴露峰值(Cmax)和达到暴露峰值的时间(Tmax)。在一些情况下，食物影响总暴露，或浓度-时间曲线下面积(AUC)。在一些实施方案中，相比于与食物一起施用恩替司他时的Cmax，在无食物情况下施用恩替司他时的Cmax较高。在一些实施方案中，在禁食情况下施用恩替司他后的Cmax与在进食情况下施用恩替司他后的Cmax之比为至少约2:1。在一个实施方案中，在禁食情况下施用恩替司他后的Cmax与在进食情况下施用恩替司他后的Cmax之比为至少约3:1。在一个实施方案中，在禁食情况下施用恩替司他后的Cmax与在进食情况下施用恩替司他后的Cmax之比为至少约4:1。在一个实施方案中，在禁食情况下施用恩替司他后的Cmax与在进食情况下施用恩替司他后的Cmax之比为至少约5:1。在一个实施方案中，在禁食情况下施用恩替司他后的Cmax与在进食情况下施用恩替司他后的Cmax之比为至少约6:1。在一个实施方案中，在禁食情况下施用恩替司他后的Cmax与在进食情况下施用恩替司他后的Cmax之比为至少约7:1。

[0068] 在一些实施方案中，相比于与食物一起施用恩替司他时的Tmax，在无食物情况下施用恩替司他时的Tmax较低。在一些实施方案中，在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约2:1。在一个实施方案中，在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约3:1。在一个实施方案中，在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约4:1。在一个实施方案中，在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约5:1。在一个实施方案中，在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约6:1。在一个实施方案中，在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约7:1。在一个实施方案中，在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约8:1。在一个实施方案中，在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约9:1。在一个实施方案中，在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约10:1。在一个实施方案中，在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约11:1。在一个实施方案中，在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约12:1。在一个实施方案中，在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约13:1。在一个实施方案中，在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约14:1。在一个实施方案中，在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约15:1。

[0069] 在一些实施方案中，在与恩替司他施用当日不同的时间施用依西美坦。在一个实施方案中，餐后施用依西美坦。在一个实施方案中，随餐施用依西美坦。

[0070] 另一个实施方案提供了这样的方法，其中在约2天治疗、约5天治疗、约7天治疗、约15天治疗或约21天治疗后测定所述疗程中蛋白质赖氨酸乙酰化水平变化。

[0071] 另一个实施方案提供了这样的方法，其中所述蛋白质赖氨酸乙酰化水平从选自B细胞、T细胞或单核细胞的组织样品获得。

[0072] 另一个实施方案提供了这样的方法，其中所述芳香酶抑制剂为依西美坦。另一个实施方案提供了这样的方法，其中所述芳香酶抑制剂为阿那曲唑。另一个实施方案提供了这样的方法，其中所述芳香酶抑制剂为来曲唑。另一个实施方案提供了这样的方法，其中每日施用所述芳香酶抑制剂。另一个实施方案提供了这样的方法，其中所述芳香酶抑制剂为依西美坦并每日施用。另一个实施方案提供了这样的方法，其中在28-天周期中每7天施用恩替司他。另一个实施方案提供了这样的方法，其中在28-天周期中每14天施用恩替司他。另一个实施方案提供了这样的方法，其中所述恩替司他-芳香酶抑制剂联合治疗包括在28-天周期中每7天口服施用恩替司他并每日口服施用依西美坦。另一个实施方案提供了这样的方法，其中所述恩替司他-芳香酶抑制剂联合治疗包括在28-天周期中每14天口服施用恩替司他并每日口服施用依西美坦。另一个实施方案提供了这样的方法，其中在28-天周期中每7天向禁食患者施用恩替司他。另一个实施方案提供了这样的方法，其中在28-天周期中每14天向禁食患者施用恩替司他。另一个实施方案提供了这样的方法，其中恩替司他-芳香酶抑制剂联合治疗包括在28-天周期中每7天向禁食患者口服施用恩替司他，并且每日口服施用依西美坦。另一个实施方案提供了这样的方法，其中恩替司他-芳香酶抑制剂联合治疗包括在28-天周期中每14天向禁食患者口服施用恩替司他，并且每日口服施用依西美坦。

[0073] 另一个实施方案提供了这样的方法，其中在疗程中进行多于一次测定蛋白质赖氨

酸乙酰化水平的步骤。另一个实施方案提供了这样的方法，其中在疗程中进行一次测定蛋白质赖氨酸乙酰化水平的步骤。

[0074] 另一个实施方案提供了这样的方法，其还包括如果在疗程中蛋白质赖氨酸乙酰化水平增加，则选择该患者以进一步治疗。

[0075] 另一个实施方案提供了这样的方法，其还包括如果在第一周疗程中蛋白质赖氨酸乙酰化水平增加，则选择该患者以进一步治疗。另一个实施方案提供的方法还包括如果在第一周和第二周疗程中蛋白质赖氨酸乙酰化水平增加，则选择该患者以进一步治疗。

[0076] 一个实施方案提供了选择患者进行进一步恩替司他-芳香酶抑制剂联合疗法的方法，其包括将开始治疗后得到的组织样品中蛋白质赖氨酸乙酰化水平与开始治疗前测定的蛋白质赖氨酸乙酰化水平比较。

[0077] 一个实施方案提供了选择患者进行进一步恩替司他-芳香酶抑制剂联合疗法的方法，其包括将开始治疗后得到的组织样品中蛋白质赖氨酸乙酰化水平与开始治疗前测定的蛋白质赖氨酸乙酰化水平比较，其中开始治疗后蛋白质赖氨酸乙酰化水平增加指示该患者将会受益于进一步治疗。

[0078] 另一个实施方案提供了这样的方法，其中超过一次测定开始治疗后得到的组织样品中蛋白质赖氨酸乙酰化水平。另一个实施方案提供了这样的方法，其中开始治疗后蛋白质赖氨酸乙酰化水平增加持续一周。另一个实施方案提供了这样的方法，其中在第2、8和15天测定开始治疗后蛋白质赖氨酸乙酰化水平。

[0079] 另一个实施方案提供了这样的方法，其中增加量为约10％到约500％。另一个实施方案提供了这样的方法，其中增加量为约10％到约400％。另一个实施方案提供了这样的方法，其中增加量为约10％到约300％。另一个实施方案提供了这样的方法，其中增加量为约10％到约200％。另一个实施方案提供了这样的方法，其中增加量为约10％到约100％。另一个实施方案提供了这样的方法，其中增加量为约10％、约20％、约30％、约40％、约50％或约60％。另一个实施方案提供了这样的方法，其中增加量为约25％、约50％、约75％、约100％、约125％或约150％。

[0080] 另一个实施方案提供了这样的方法，其中所述组织样品选自B细胞、T细胞或单核细胞。

[0081] 另一个实施方案提供了这样的方法，其中开始治疗后得到的组织样品是在开始治疗至少2天后获得的。另一个实施方案提供了这样的方法，其中所述开始治疗后得到的组织样品是在开始治疗后第2天至第28天之间获得的。另一个实施方案提供了这样的方法，其中所述开始治疗后得到的样品是在开始治疗后第2、3、4、5、6、7、8、9、10、11、12、13、14或15天获得的。

[0082] 一个实施方案提供了选择患者进行进一步恩替司他-芳香酶抑制剂联合疗法的方法，其包括将开始治疗后得到的组织样品中蛋白质赖氨酸乙酰化水平与开始治疗前测定的蛋白质赖氨酸乙酰化水平的变化百分比比较，其中开始治疗后蛋白质赖氨酸乙酰化水平降低百分比为约5％至约50％指示该患者将不会受益于进一步治疗。

[0083] 一个实施方案提供了治疗对之前的芳香酶抑制剂疗法显示抗性的乳腺癌的方法，所述方法包括向患者施用包含恩替司他和芳香酶抑制剂的组合，其中所述患者在之前的芳香酶抑制剂治疗过程中未显示完全应答、部分应答或大于6个月的病情稳定。在一些情况

下,向禁食患者施用恩替司他。

[0084] 另一个实施方案提供了这样的方法,其中所述患者在之前作为辅助疗法给予的非甾体芳香酶抑制剂治疗过程中或结束的6个月内复发。

[0085] 另一个实施方案提供了这样的方法,其中所述患者在之前非甾体芳香酶抑制剂治疗至少3个月后显示疾病进展。

[0086] 另一个实施方案提供了这样的方法,其中所述乳腺癌为ER阳性。

[0087] 另一个实施方案提供了这样的方法,其中所述与恩替司他联合施用的芳香酶抑制剂为来曲唑。另一个实施方案提供了这样的方法,其中所述与恩替司他联合施用的芳香酶抑制剂为阿那曲唑。另一个实施方案提供了这样的方法,其中所述与恩替司他联合施用的芳香酶抑制剂为依西美坦。

[0088] 另一个实施方案提供了这样的方法,其中按顺序依次施用或同时施用恩替司他和所述芳香酶抑制剂。另一个实施方案提供了这样的方法,其中同时施用恩替司他和所述芳香酶抑制剂。另一个实施方案提供了这样的方法,其中首先施用所述芳香酶抑制剂。另一个实施方案提供了这样的方法,其中每日施用所述芳香酶抑制剂并周期性施用恩替司他。另一个实施方案提供了这样的方法,其中每周施用恩替司他并每日施用所述所述芳香酶抑制剂。另一个实施方案提供了这样的方法,其中将恩替司他引入正在进行的芳香酶抑制剂疗程中。

[0089] 另一个实施方案提供了这样的方法,其还包括除恩替司他和选自来曲唑、阿那曲唑或依西美坦或其药学可接受的盐、溶剂合物或前药的芳香酶抑制剂的组合外还向受试者施用一种或多种疗法。

[0090] 另一个实施方案提供了这样的方法,其中所述一种或多种疗法包括放疗、化疗、高剂量化疗伴干细胞移植以及单克隆抗体疗法中的一种或多种。另一个实施方案提供了这样的方法,其中所述放疗包括体内和/或体外放疗。另一个实施方案提供了这样的方法,其中所述化疗包括向受试者施用多柔比星、环磷酰胺、紫杉醇、拉帕替尼、卡培他滨、曲妥珠单抗、贝伐珠单抗、吉西他滨、艾瑞布林或白蛋白结合型紫杉醇(nab-paclitaxel)中的一种或多种。

[0091] 一个实施方案提供了治疗有需要的患者的乳腺癌的方法,该方法包括口服施用依西美坦和恩替司他,其中恩替司他施用于禁食患者。另一个实施方案提供了治疗乳腺癌的方法,其中施用后恩替司他Tmax少于1小时。另一个实施方案提供了治疗乳腺癌的方法,其中施用后恩替司他Tmax少于90分钟。

另一个实施方案提供了治疗乳腺癌的方法,其中施用后恩替司他Tmax少于2小时。另一个实施方案提供了治疗乳腺癌的方法,其中施用后恩替司他Tmax为30分钟至2小时。另一个实施方案提供了治疗乳腺癌的方法,其中口服施用恩替司他后,恩替司他Cmax为至少150ng/mL。另一个实施方案提供了治疗乳腺癌的方法,其中口服施用恩替司他后,恩替司他Cmax为至少125ng/mL。另一个实施方案提供了治疗乳腺癌的方法,其中口服施用恩替司他后,恩替司他Cmax为至少100ng/mL。

另一个实施方案提供了治疗乳腺癌的方法,其中口服施用恩替司他后,恩替司他Cmax为至少80ng/mL。另一个实施方案提供了治疗乳腺癌的方法,其中口服施用恩替司他后,恩替司他Cmax为至少50ng/mL。另一个实施方案提供了治疗乳腺癌的方法,其中施用约5mg的

恩替司他。另一个实施方案提供了治疗乳腺癌的方法，其中施用约10mg的恩替司他。另一个实施方案提供了治疗乳腺癌的方法，其中施用约1mg至约20mg的恩替司他。另一个实施方案提供了治疗乳腺癌的方法，其中每周一次施用恩替司他。另一个实施方案提供了治疗乳腺癌的方法，其中在28-天周期内施用恩替司他。另一个实施方案提供了治疗乳腺癌的方法，其中所述患者在施用恩替司他前2小时内不进食。另一个实施方案提供了治疗乳腺癌的方法，其中所述患者在施用恩替司他前1小时内不进食。

[0092] 另一个实施方案提供了治疗乳腺癌的方法，其中所述患者在施用恩替司他后2小时内不进食。另一个实施方案提供了治疗乳腺癌的方法，其中所述患者在施用恩替司他后30分钟内不进食。另一个实施方案提供了治疗乳腺癌的方法，其中在与恩替司他施用当日不同的时间施用依西美坦。另一个实施方案提供了治疗乳腺癌的方法，其中餐后施用依西美坦。另一个实施方案提供了治疗乳腺癌的方法，其中随餐施用依西美坦。另一个实施方案提供了治疗乳腺癌的方法，其中每日一次施用约25mg的依西美坦。

肺癌的治疗

表皮生长因子受体

[0093] 在过去的几年中，关于与癌症行为相关的分子机制和细胞转化的知识已经得以增加。自从针对涉及许多类型癌症的致癌作用的过程的特定靶向治疗开发以来，已经产生了更多的兴趣。在90年代，发现表皮生长因子受体(EGFR)在肿瘤样生物学和行为中发挥重要作用。EGFR刺激使影响细胞增殖和活动、血管生成和其他机制的细胞内信号传导和级联活化。正常细胞受到外部因素影响，在肿瘤细胞中发现由该受体介导的细胞增殖的活化将不再需要外部刺激，而是独立自主地起作用。在NSCLC的情况下，已表明该受体的过度表达以及特定的体细胞突变发生在它们的具有酪氨酸激酶活性的细胞内结构域(外显子18与21之间)中，其可影响预后，与阶段、存活和化疗反应显著相关。这些数据导致各种物质的开发和研究，包括针对EGFR的细胞外结构域的单克隆抗体(例如，西妥昔单抗、Erbitux®)和抑制EGFR的酪氨酸激酶细胞内结构域(酪氨酸激酶抑制剂，TKI)的小分子(例如，吉非替尼和厄洛替尼)。采用这些TKI进行的随机化临床试验的初步结果已经证明它们在患有晚期疾病的患者中的使用是有效的，从而显著提高了这些患者的存活率，尤其当他们具有更常发现于具有腺癌组织学亚型(尤其是在细支气管肺泡癌的存在下)的亚洲族群的不吸烟、女性患者的亚群中的EGFR的突变时。这些结果中的一些如此令人印象深刻，以致于该现象被称为Lazarus效应，并导致厄洛替尼在美国和欧洲被批准用于NSCLC患者的第二和第三线治疗；以及吉非替尼在欧洲被批准用于具有EGFR突变的患者(del Mello等人，World J Clinc Oncol，Vol.2，p.367(2011))。

[0094] EGFR也称为ErbB1或Her1，是由位于染色体7上的基因(7p12.1-12.3)编码的跨膜糖蛋白。EGFR包含1186个氨基酸(a.a.)和26个外显子。外显子1-14编码细胞外结构域，外显子15编码跨膜区，且外显子16-26编码细胞内结构域。该糖蛋白属于ErbB受体家族，该家族还包括：ErbB2(HER2/neu)、ErbB3(HER3)和ErbB4(HER4)。这些蛋白质中的每一种在结构上均由细胞外结构域、疏水性跨膜结构域和具有固有酪氨酸激酶(TK)活性的细胞内结构域组成(除了ErbB3)。这些受体作为非活性的单体存在，被它们通过细胞外结构域与EGF家族的生长因子的相互作用所活化。ErbB受体分子与这些配体中的一个结合导致它与同一家族的其他单体相互作用(受体二聚)。该二聚可发生在两个相同的受体之间(同源二聚化，例如

ErbB1-ErbB1)或两个不同的受体之间(异源二聚化,例如ErbB1-ErbB3)。由特异性配体引起的刺激引发独特模式的二聚,其对于发生该现象的组织/肿瘤也具有特异性。这些受体的二聚导致它们的自身磷酸化,以及TK的活化和调节这样的多种活性如增殖、分化、凋亡和细胞迁移的细胞内生化过程的级联的活化。

E钙粘蛋白

[0095] 上皮钙粘蛋白(E钙粘蛋白)也称为钙粘蛋白-1、CAM 120/80或桑椹粘着蛋白,是由CDH1基因编码的人类中的蛋白质。E钙粘蛋白是钙粘蛋白超家族的典型成员。E钙粘蛋白是钙依赖性细胞-细胞粘附糖蛋白,其由细胞外结构域中的五个细胞外钙粘蛋白重复(EC1-EC5)、跨膜结构域、与p120-连环蛋白和β-连环蛋白结合的细胞内结构域以及高度保守的胞质尾区组成。细胞内结构域含有对β-连环蛋白结合至关重要以及因此对E钙粘蛋白功能至关重要的高度磷酸化区。β-连环蛋白还可与α-连环蛋白结合。α-连环蛋白参与含肌动蛋白的细胞骨架丝的调节。在上皮细胞中,含E钙粘蛋白的细胞至细胞的连接通常与含肌动蛋白的细胞骨架丝相邻。

[0096] 这种基因中的突变与胃癌、乳腺癌、结直肠癌、甲状腺癌和卵巢癌相关。功能或表达的缺失被认为有助于癌症和转移的进展。E钙粘蛋白下调降低了组织内的细胞粘附强度,从而导致细胞运动性增加。这反过来可能允许癌细胞跨越基底膜并侵入周围组织。

测定E钙粘蛋白水平的方法

[0097] E钙粘蛋白水平可以通过ELISA进行定量测量。一些E钙粘蛋白ELISA试剂盒诸如由TaKaRA提供的E钙粘蛋白EIA试剂盒是固相夹心EIA,其利用两种小鼠单克隆E钙粘蛋白抗体(其中一种涂覆在板上,而另一种用POD标记)来使用两步温育法检测人E钙粘蛋白。在第一步中,在抗体涂覆的微量滴定板中温育样品。在第二步的过程中,洗涤该板并将其与POD标记的E钙粘蛋白抗体一起温育。添加底物,并且POD与底物(H2O2、TMBZ)之间的反应导致颜色形成。样品可溶性E钙粘蛋白的量通过使用EIA平板阅读器测量吸光度来测定。准确的可溶性E钙粘蛋白样品浓度可通过将它们特定的吸光度与获得的绘制在标准曲线上的标准吸光度进行比较来测定。在一些实施方案中,E钙粘蛋白水平通过ELISA进行定量测量。

[0098] E钙粘蛋白水平可以通过免疫组织化学进行检测。为了检测在浸没固定细胞中的E钙粘蛋白水平,将细胞与10μg/mL的人E钙粘蛋白抗原亲和力纯化的多克隆抗体(R&D Systems®目录号AF648)在室温下一起温育3小时。然后使用NorthernLights™ 557偶联的抗山羊IgG二级抗体(R&D Systems®目录号NL001)将细胞染色并用DAPI复染。可使用荧光显微镜将E钙粘蛋白和DAPI可视化,并且滤光器设置为适合所用的标记物。在一些实施方案中,E钙粘蛋白水平通过免疫组织化学进行检测。

[0099] E钙粘蛋白水平可以通过免疫细胞化学进行检测。用于免疫细胞化学(ICC)的盖玻片可使用明胶制备。在一些实施方案中,用于制备ICC的盖玻片的方法包括a)将已灭菌的盖玻片放置于24孔板的孔中,b)加入400μL的明胶涂覆溶液,以及c)在室温下温育盖玻片10分钟。然后除去明胶涂覆溶液并将盖玻片风干15分钟。经干燥的盖玻片可在室温下储存直至使用。一旦制备好盖玻片,则可按如下制备并固定细胞。通过向含有明胶涂覆的盖玻片的细胞培养板的孔中添加500μL含约5000个细胞的培养基来培养细胞。当细胞达到所需的密度/期时,从每个孔中去除培养基并用PBS洗涤两次。向每个孔中添加300-400μL的2-4%甲醛固

定溶液，并在室温下温育20分钟。将孔用PBS洗涤两次，并用400μL的洗涤缓冲液覆盖。可将盖玻片在2-8℃下储存最长3个月或可将其立即染色。一旦制备好细胞，可按如下将细胞染色用于ICC。将含有固定的细胞的盖玻片在400μL的洗涤缓冲液中洗涤两次。通过添加400μL封闭缓冲液阻断非特异性染色，并在室温下温育45分钟。去除封闭缓冲液。不必冲洗。根据制造商的说明在稀释缓冲液中稀释未偶联的第一抗体(或荧光偶联的第一抗体)。对于使用R&D Systems抗体对盖玻片上的细胞进行荧光ICC染色，建议在室温下温育1小时。或者，在2-8℃下温育过夜。在400μL的洗涤缓冲液中洗涤两次。如果使用具有直接荧光偶联物的第一抗体，则转到步骤8。根据制造商的说明在稀释缓冲液中稀释第二抗体。向孔中加入400μL，并在黑暗中在室温下温育1小时。从该步骤开始，样品应当避光。在400μL的洗涤缓冲液中冲洗两次。向每个孔中添加300μL经稀释的DAPI溶液，并在室温下温育2-5分钟。DAPI与DNA结合，并且是方便的核复染剂。它在358nm处具有最大吸收并且荧光蓝在461nm处具有发射最大值。用PBS冲洗一次并用水冲洗一次。从孔中小心地取出盖玻片并吸干以去除任何过量的水。向每个盖玻片的显微镜载玻片上分配1滴抗荧光淬灭封片液(anti-fade mounting medium)。用面朝显微镜载玻片的细胞覆盖盖玻片。使用荧光显微镜和适合所用的标记物的滤光器组进行可视化。还可将载玻片在<-20℃下储存于载玻片盒中，以用于后续的考查。在一些实施方案中，E钙粘蛋白水平通过免疫细胞化学进行检测。

[0100] E钙粘蛋白基因表达可通过测量E钙粘蛋白甲基化进行测定。E钙粘蛋白甲基化试剂盒诸如由Millipore®提供的CpGWIZ®E钙粘蛋白扩增试剂盒，通过甲基化特异性PCR(MSP)测定E钙粘蛋白启动子的甲基化状态。该试剂盒含有靶向启动子区的引物，在该启动子区中序列在亚硫酸氢盐处理后呈现最大差异。确定PCR参数，使得试剂盒中的所有引物组在相同的条件下扩增。针对E钙粘蛋白的对照基因组DNA样品(甲基化的和未甲基化的)也包含在内。在一些实施方案中，E钙粘蛋白基因表达通过测量E钙粘蛋白甲基化进行测定。

[0101] 一个实施方案提供了治疗在先前治疗后进展的未使用过EGFR抑制剂的患者的癌症的方法，其中所述患者表现出高E钙粘蛋白表达水平，该方法包括向患者施用包含恩替司他和EGFR抑制剂的组合。另一个实施方案提供了这样的方法，其中高E钙粘蛋白表达水平通过ELISA、免疫组织化学、免疫细胞化学或测定E钙粘蛋白甲基化水平进行表征。另一个实施方案提供了其中高E钙粘蛋白表达水平通过免疫组织化学进行测定的方法。另一个实施方案提供了这样的方法，其中高E钙粘蛋白表达水平得分为+3，如通过免疫组织化学测定的。

肺癌

[0102] 在美国和全世界肺癌都是女性和男性癌症死亡的主要原因。肺癌已超越乳腺癌成为女性癌症死亡的主要原因。预计2010年在美国有157,300人死于肺癌，这高于由结肠癌和直肠癌、乳腺癌以及前列腺癌总共导致的死亡数。尽管在最早的阶段诊断的肺癌的存活率较高，约49％存活五年或更长时间，但被诊断为患有已经扩散至身体其他部位的肺癌的这些人中仅约2％在诊断后存活五年。

[0103] 当正常细胞经历导致其不受控制地生长和繁殖的转变时癌症发生。该细胞形成不同于其所出现的周围组织的肿块或肿瘤。肿瘤是危险的，因为它们耗费氧、营养物和健康细胞的空间并且因为它们侵入并破坏或降低正常组织起作用的能力。

[0104] 大多数肺部肿瘤是恶性的。这意味着它们侵入并破坏其周围的健康组织，并可扩散至全身。肿瘤可扩散至附近的淋巴结或通过血流扩散至其他器官。该过程被称为转移。当

肺癌转移时，肺部的肿瘤被称为原发性肿瘤，而身体其他部位的肿瘤被称为继发性肿瘤或转移性肿瘤。

[0105] 肺部的一些肿瘤是从身体的其他部位的癌症转移的。肺是转移的常见部位。如果是这种情况，则该癌症不被认为是肺癌。例如，如果前列腺癌通过血流扩散至肺，则它是在肺中的转移性前列腺癌(继发性癌症)，而不被称为肺癌。

[0106] 肺癌包括一组不同类型的肿瘤。肺癌通常被分为两个主要的组，其占所有病例的约95％。这些组的划分是基于组成癌症的细胞类型。肺癌的两种主要类型通过在显微镜下观察时肿瘤的细胞大小来表征。它们被称为小细胞肺癌(SCLC)和非小细胞肺癌(NSCLC)。NSCLC包括几种肿瘤亚型。SCLC不太常见，但其生长较快并且比NSCLC更有可能转移。通常，当诊断到癌症时SCLC已经扩散至身体的其他部位。约5％的肺癌具有罕见的细胞类型，包括类癌瘤、淋巴瘤及其他。如本文所用的术语“肺癌”包括但不限于，SCLC、NSCLC、类癌瘤、淋巴瘤和它们的各种亚型。

非小细胞肺癌

[0107] NSCLC是一种肺部癌症，它不是小细胞癌(燕麦细胞癌)类型。术语“非小细胞肺癌”适用于各种类型的支气管癌(从支气管内层产生的那些癌)。特定类型的NSCLC的实例包括但不限于，腺癌、鳞状细胞癌和大细胞癌(即，大细胞未分化癌)。

[0108] 腺癌是在器官的内层或内表面形成的癌症。腺癌是肺癌的最常见类型，其占所有肺癌病例的30％-40％。腺癌的一种亚型被称为支气管肺泡细胞癌，其在胸部X射线下产生类似肺炎的外观。

[0109] 鳞状细胞癌是开始于鳞状细胞的癌症。鳞状细胞是在显微镜下看起来像鱼鳞的薄平细胞。鳞状细胞发现于形成皮肤表面、身体中空器官的内层以及呼吸道和消化道的通道的组织中。鳞状细胞癌可出现在这些组织中的任何组织中。鳞状细胞癌是肺癌的第二个最常见类型，占全部病例的约30％。

[0110] 大细胞癌不显示鳞状或腺成熟的迹象。因此，这些肿瘤通常进行默认诊断(当已经排除所有其他的可能性时)。在活检之前这些肿瘤缺乏表明其诊断的任何诊断特征。它们倾向于生长迅速、早期转移，并与吸烟密切相关。大细胞肿瘤通常是具有广泛性出血和坏死的大的、蓬松的、边界清楚的、粉灰色肿块。尽管它们通常具有中心坏死，但它们很少空化。它们倾向于存在于中间至外围的肺区域。它们可局部扩展以包括段支气管或亚段支气管。大细胞癌的变体是巨细胞癌。该亚型尤其具有攻击性，并且携带极差的预后。这些肿瘤通常作为具有局灶性坏死部分的大的周边肿块存在。它们不包括大的气道，除非通过直接扩展。大细胞癌占所有肺癌病例的10％-20％。

小细胞肺癌

[0111] SCLC也被称为燕麦细胞肺癌并且是肺癌的一种类型，其中细胞在显微镜下看起来小且圆。SCLC由于其临床和生物学特征而被认为不同于其他肺癌。小细胞肺癌表现出攻击行为，且生长迅速、早期扩散至远处部位、对化疗和辐射极为敏感，且常与不同的副癌综合征相关。小细胞癌出现在支气管周围的位置并浸润支气管粘膜下层。广泛的转移发生在疾病过程的早期，常扩散至纵隔淋巴结、肝、骨骼、肾上腺和脑。此外，各种肽激素的产生导致宽范围的副癌综合征；这些中最常见的是抗利尿激素不适当分泌综合征(SIADH)和异位促肾上腺皮质激素(ACTH)综合征产生。此外，自身免疫现象可导致各种神经综合征，诸如兰伯

特-伊顿(Lambert-Eaton)综合征。SCLC占所有病例的20％。类癌瘤

[0112] 类癌瘤是分泌大量激素血清素的肿瘤。类癌瘤也被称为嗜银细胞瘤。该肿瘤通常出现在胃肠道中，即胃与直肠之间的任何地方(最喜欢的地方在阑尾中)并可从这里转移至肝。在肝中该肿瘤产生并释放大量的血清素至全身的血流。该结果被称为类癌综合征。它由血清素直接引起，并包括潮红和脸红、脸(特别是眼睛周围)的肿胀、皮肤上的扁平状血管瘤(扩张血管的小的聚集)、腹泻、支气管痉挛、数脉、低血压以及三尖瓣和肺动脉狭窄(心脏的三尖瓣和肺动脉瓣变窄)，常伴随回流。以下四种类型的治疗中的一种或多种用于类癌瘤：手术(取出癌症)；放疗(使用高剂量X射线杀死癌细胞)；生物治疗(使用身体的自然免疫系统对抗癌症)；以及化疗(使用药物杀死癌细胞)。由于类癌瘤分泌激素(血清素)，因此其被认为是一种内分泌肿瘤类型。类癌瘤可作为某些遗传病症诸如多发性内分泌瘤(MEN)1型和神经纤维瘤1型(NF1或冯.雷克林豪森(von Recklinghausen)病)的一部分而发生。类癌瘤占所有病例的1％。

淋巴瘤

[0113] 淋巴瘤是涉及免疫系统的细胞(被称为淋巴细胞)的癌症类型，并主要代表参与身体的淋巴系统的细胞。淋巴瘤是B细胞或T细胞或其亚型的恶性转化。淋巴瘤归入两种主要类型中的一种：霍奇金淋巴瘤(HL，先前被称为霍奇金病)以及所有其他淋巴瘤(非霍奇金淋巴瘤或NHL)。这两种类型发生在相同的位置，可能与相同的症状相关，并通常在身体检查时具有类似的外观。然而，它们通过显微镜检查可容易地区分。霍奇金病由特定的异常B淋巴细胞谱系形成。NHL可源自异常B或T细胞并通过独特的遗传标志物区别。有五种霍奇金病亚型和约30种非霍奇金淋巴瘤亚型。因为有如此多的不同的淋巴瘤亚型，因此淋巴瘤的分类是复杂的(其包括显微镜外观以及遗传和分子标志物)。许多NHL亚型看起来相似，但它们在功能上差异较大并响应于具有不同治愈概率的不同治疗。HL亚型在显微镜下是不同的，并且基于显微镜差异以及疾病的程度进行分类。

EGFR抑制剂

[0114] EGFR抑制剂通过靶细胞中的表皮生长因子受体(EGFR)来中断信号传导。某些EGFR抑制剂诸如厄洛替尼已被批准用于治疗转移性NSCLC。对于晚期NSCLC，EGFR抑制剂诸如吉非替尼已经被批准。若干更多的EGFR抑制剂在临床试验中进行测试以用于治疗NSCLC和其他肺癌。

[0115] 如本文所述的“EGFR抑制剂”是抑制EGF受体活性的分子。化合物(EGFR的抑制剂)可由本领域技术人员使用方法例如测量由激酶反应形成的ADP的EGFR激酶测定容易地鉴定。

[0116] 已对作为肺癌的治疗选择的EGFR的抑制进行了研究，并获得了一些成功。目前的三种EGFR抑制剂，即厄洛替尼、吉非替尼和西妥昔单抗，在美国被批准销售以用于治疗肺癌。

[0117] 厄洛替尼(Tarceva ®)被批准用于治疗转移性非小细胞肺癌和不能通过手术移除或已经转移的胰腺癌。该小分子药物抑制EGFR的酪氨酸激酶活性。

[0118] 吉非替尼(Iressa®)被批准用于治疗患有晚期非小细胞肺癌的患者。该小分子药物被限制在这样的患者中使用，在该患者的治疗医生看来其目前正受益于或先前已受益于

吉非替尼治疗。吉非替尼抑制表皮生长因子受体(EGFR)的酪氨酸激酶活性，该表皮生长因子受体通过许多类型的癌细胞过度产生。

[0119] 西妥昔单抗(Erbitux ®)是单克隆抗体，其被批准用于治疗患有头颈癌或结直肠癌的鳞状细胞癌的一些患者。该治疗与EGFR的外部结合，从而防止受体被生长信号活化，这可抑制信号转导并导致抗增殖效果。

[0120] EGFR抑制剂的其他实例包括但不限于，帕尼单抗、凡德他尼、拉帕替尼、卡奈替尼、阿法替尼、奈昔木单抗(necitumumab)、尼妥珠单抗、PF299804、RO5083945、ABT-806和AP26113。

[0121] 帕尼单抗(Vectibix ®)被批准用于治疗患有转移性结肠癌的一些患者。该单克隆抗体附着于EGFR并阻止其发出生长信号。

[0122] 凡德他尼(Caprelsa®)被批准用于治疗患有转移性甲状腺髓样癌的不适合手术的患者。该小分子药物结合并阻断若干酪氨酸激酶，包括EGFR、血管内皮生长因子受体(VEGF)的若干受体和RET的生长促进活性。

[0123] 拉帕替尼(Tykerb ®)被批准用于治疗某些类型的晚期或转移性乳腺癌。该小分子药物抑制若干酪氨酸激酶，包括HER-2的酪氨酸激酶活性。拉帕替尼治疗阻止HER-2信号激活细胞生长。

[0124] 卡奈替尼是口服生物可用的不可逆的泛ErbB酪氨酸激酶抑制剂，靶向EGFR、HER-2、ErbB-3和ErbB-4。它有效地抑制了食管鳞状细胞癌(其共表达EGFR和HER2)的生长，并抑制MAPK和AKT的磷酸化。人类癌细胞系体外研究表明卡奈替尼导致对酪氨酸激酶活性的迅速强力且持久的抑制。

[0125] 阿法替尼是不可逆的EGFR/HER2。在无细胞体外激酶测定中，阿法替尼显示了对野生型和突变型EGFR和HER2的强力活性，其类似于吉非替尼对L858R EGFR的效能，但针对吉非替尼抗性L858R-T790M EGFR双突变体比吉非替尼的活性高约100倍。阿法替尼在抑制具有野生型(H1666)或L858R/T790M(NCI-H1975)EGFR的肺癌细胞系的存活中是有效的。在表皮样癌细胞系A431的标准异种移植模型中进行评价。用20mg/kg阿法替尼日常口服治疗25天导致显著的肿瘤消退，且累计治疗的/对照肿瘤体积比(T/C比)为2％。如同拉帕替尼和来那替尼，阿法替尼是下一代酪氨酸激酶抑制剂(TKI)，其不可逆地抑制人类表皮生长因子受体2(Her2)和表皮生长因子受体(EGFR)激酶。阿法替尼不仅对由第一代TKI如厄洛替尼或吉非替尼靶向的EGFR突变有活性，而且针对那些对这些标准治疗不敏感的突变也有活性。由于阿法替尼对Her2的附加活性，其被研究用于乳腺癌以及其他EGFR和Her2驱动的癌症。

[0126] 奈昔木单抗是针对具有潜在抗肿瘤活性的表皮生长因子受体(EGFR)的完整人类IgG1单克隆抗体。奈昔木单抗结合并阻断EGFR的配体结合位点，从而阻止受体的活化以及后续的二聚。这可能导致对依赖EGFR的下游途径的抑制并因此抑制依赖EGFR的肿瘤细胞增殖和转移。

[0127] 尼妥珠单抗是针对具有潜在抗肿瘤活性的表皮生长因子受体(EGFR)的人源化单克隆抗体。尼妥珠单抗结合并抑制EGFR，从而导致对过度表达EGFR的肿瘤细胞的生长抑制。该药剂可与放疗协同作用。

[0128] PF299804是人类表皮生长因子受体(HER)-1/EGFR、-2和-4酪氨酸激酶(TK)的强力不可逆抑制剂,它在E敏感和E抗性的临床前模型中是活性的。在I/II期试验中PF299804在EGFR TK抑制剂(TKI)难治的NSCLC中具有临床活性。

[0129] RO5083945是对在免疫效应细胞上表达的所有Fc γ RIIIa变体表现出增加的结合亲和力的糖工程化的(glycoengineered)抗EGFR IgG1 mAb。RO5083945在基于ADCC的测定中显示了显著改善的细胞杀伤性,并与西妥昔单抗和帕尼单抗相比在体内模型中显示了较大的活性。因此,RO5083945具有在患有实体肿瘤包括KRAS突变型CRC的患者中表现出临床活性的潜力。

[0130] ABT-806是针对具有抗肿瘤活性的人类表皮生长因子受体(EGFR)的人源化单克隆抗体(MoAb)。MoAb ABT-806靶向EGFR缺失变体、de2-7 EGFR以及在过度表达受体的细胞中表达的野生型EGFR,从而阻止受体的活化以及后续的二聚;受体活化和二聚的减少导致对信号转导的抑制和抗增殖效果。该MoAb靶向表达异常EGFR的细胞,从而使得它成为用于生成放射性同位素或毒素偶合物的理想候选物。

[0131] AP26113是受体酪氨酸激酶间变性淋巴瘤激酶(ALK)和具有潜在抗肿瘤活性的表皮生长因子受体(EGFR)的口服可用抑制剂。双ALK/EGFR抑制剂AP26113结合并抑制ALK激酶和ALK融合蛋白质以及EGFR和突变型。这导致对ALK激酶和EGFR激酶的抑制,破坏它们的信号传导途径并最终抑制敏感的肿瘤细胞中的肿瘤细胞生长。此外,AP26113似乎克服了基于突变的抗性。ALK属于胰岛素受体超家族,并在神经系统发育中发挥重要作用;ALK调节异常和基因重排与一系列的肿瘤相关。EGFR在多种癌细胞类型中过度表达。

其他治疗

[0132] 可有利地与本文公开的治疗联合使用的其他可用的肺癌治疗包括但不限于放疗、化疗、抗体治疗和酪氨酸激酶抑制剂作为辅助治疗。

[0133] 放疗是一种采用高能x射线或其他类型的辐射杀死癌细胞或防止其生长的癌症治疗。化疗是一种采用药物来通过杀死细胞或通过阻止其分裂而阻止癌细胞生长的癌症治疗。当化疗被口服或注射到静脉或肌肉中时,药物进入血流并可到达全身的癌细胞(全身化疗)。当化疗直接置于脊柱、器官或体腔如腹腔中时,药物主要影响那些区域中的癌细胞(区域化疗)。给予化疗的方式取决于被治疗的癌症的类型和阶段。

[0134] 用于治疗肺癌的不同化疗剂是本领域已知的。用于治疗肺癌的细胞毒性剂包括卡铂(例如,Paraplatin®、Paraplat®)、顺铂(例如,Platinol®、Platinol-Aq®)、克唑替尼(例如,Xalkori®)、依托泊苷(例如Toposar®、VePesid®)、磷酸依托泊苷(例如,Etopophos®)、盐酸吉西他滨(例如,Gemzar®)、吉西他滨-顺铂、甲氨蝶呤(例如,Abitrexate®、Folex®、Folex PFs®、Methotrexate Lpf®、Mexate®、Mexate-Aq®)、紫杉醇(例如Taxol®)、培美曲塞二钠(例如,Alimta®)和盐酸拓扑替康(例如Hycamtin®)。

[0135] 单克隆抗体治疗是一种使用在实验室中由单个类型的免疫系统细胞制备的抗体的癌症治疗。这些抗体可识别癌细胞上的物质或可以帮助癌细胞生长的正常物质。该抗体附于所述物质并杀死癌细胞,阻断其生长或防止其扩散。单克隆抗体通过输注给予。单克隆

抗体可单独使用或用于将药物、毒素或放射性物质直接携带至癌细胞。单克隆抗体还作为辅助治疗与化疗联合使用。

[0136] 贝伐珠单抗(Avastin®)是一种针对血管内皮生长因子(VEGF)—一种促血管生成细胞因子的重组人源化单克隆抗体。贝伐珠单抗与VEGF结合并抑制VEGF受体结合,由此阻止肿瘤血管的生长和维持。贝伐珠单抗目前用于治疗若干种类型的癌症,包括结直肠癌、肺癌、乳腺癌和肾癌以及成胶质细胞瘤的某些类型。

[0137] 可有利地与本文所公开的组合物和治疗组合的其他示例性治疗可包括但不限于施用药剂,该药剂包括但不限于拉帕替尼(单独使用或与卡培他滨组合),多西紫杉醇,表柔比星,埃博霉素A、B或D,醋酸戈舍瑞林,紫杉醇,帕米膦酸二钠,贝伐珠单抗或曲妥珠单抗。

[0138] 在一些实施方案中,其他治疗包括化疗,该化疗包括向受试者施用以下的一种或多种:多柔比星、环磷酰胺、紫杉醇、拉帕替尼、卡培他滨、曲妥珠单抗、贝伐珠单抗、吉西他滨、艾瑞布林或白蛋白结合型紫杉醇。

用于治疗肺癌的方法

[0139] 一个实施方案提供了治疗在先前的治疗后进展的EGFR抑制剂初次患者的癌症的方法,其中该方法包括:(1)确定患者的E钙粘蛋白表达水平;(2)选择得分为+3的表现出高E钙粘蛋白表达水平的患者;以及(3)向患者施用包含恩替司他和EGFR抑制剂的组合。在一些情况下,向禁食患者施用恩替司他。

[0140] 在一些实施方案中,与恩替司他和食物一起施用时的Cmax相比,在无食物的情况下施用恩替司他时的Cmax较高。在一些实施方案中,在禁食情况下施用恩替司他后的Cmax与在进食情况下施用恩替司他后的Cmax之比为至少约2:1。在一个实施方案中,在禁食情况下施用恩替司他后的Cmax与在进食情况下施用恩替司他后的Cmax之比为至少约3:1。在一个实施方案中,在禁食情况下施用恩替司他后的Cmax与在进食情况下施用恩替司他后的Cmax之比为至少约4:1。在一个实施方案中,在禁食情况下施用恩替司他后的Cmax与在进食情况下施用恩替司他后的Cmax之比为至少约5:1。在一个实施方案中,在禁食情况下施用恩替司他后的Cmax与在进食情况下施用恩替司他后的Cmax之比为至少约6:1。在一个实施方案中,在禁食情况下施用恩替司他后的Cmax与在进食情况下施用恩替司他后的Cmax之比为至少约7:1。

[0141] 在一些实施方案中,与恩替司他和食物一起施用时的Tmax相比,在无食物的情况下施用恩替司他时的Tmax较低。在一些实施方案中,在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约2:1。在一个实施方案中,在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约3:1。在一个实施方案中,在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约4:1。在一个实施方案中,在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约5:1。在一个实施方案中,在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约6:1。在一个实施方案中,在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约7:1。在一个实施方案中,在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约8:1。在一个实施方案中,在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约9:1。在一个实施方案中,在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约10:1。在一个实施方案中,在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之

比为至少约11:1。在一个实施方案中，在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约12:1。在一个实施方案中，在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约13:1。在一个实施方案中，在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约14:1。在一个实施方案中，在进食情况下施用后的Tmax与在禁食情况下施用后的Tmax之比为至少约15:1。

[0142] 在一些实施方案中，在与恩替司他施用当日不同的时间施用EGFR抑制剂。在一个实施方案中，向禁食患者施用EGFR抑制剂。

[0143] 另一个实施方案提供了其中先前的治疗是一种先前的化疗的方法。

[0144] 另一个实施方案提供了其中先前的治疗是两种或更多种先前的化疗的方法。

[0145] 另一个实施方案提供了这样的方法，其中高E钙粘蛋白表达水平通过ELISA、免疫组织化学、免疫细胞化学或测定E钙粘蛋白甲基化水平进行测定。另一个实施方案提供了这样的方法，其中高E钙粘蛋白表达水平通过免疫组织化学进行测定。另一个实施方案提供了这样的方法，其中高E钙粘蛋白表达水平得分为+3，如通过免疫组织化学测定的。

[0146] 另一个实施方案提供了其中所述癌症是肺癌的方法。

[0147] 另一个实施方案提供了其中所述肺癌是非小细胞肺癌的方法。

[0148] 另一个实施方案提供了其中与恩替司他联合施用的EGFR抑制剂是厄洛替尼的方法。

[0149] 另一个实施方案提供了其中以任意顺序连续施用或同时施用恩替司他和EGFR抑制剂的方法。另一个实施方案提供了其中同时施用恩替司他和EGFR抑制剂的方法。另一个实施方案提供了其中首先施用EGFR抑制剂的方法。

[0150] 另一个实施方案提供了其中每日施用EGFR抑制剂并且周期性施用恩替司他的方法。另一个实施方案提供了其中每日施用EGFR抑制剂并且每周施用恩替司他的方法。

[0151] 另一个实施方案提供了治疗在先前的治疗后进展的EGFR抑制剂初次患者的癌症的方法，其中所述患者表现出高E钙粘蛋白表达水平，该方法包括向患者施用包含恩替司他和EGFR抑制剂的组合。

[0152] 另一个实施方案提供了治疗在先前的治疗后进展的EGFR抑制剂初次患者的癌症的方法，其中所述患者表现出高E钙粘蛋白表达水平，其中该方法还包括除了恩替司他和EGFR抑制剂的组合之外，还向受试者施用一种或多种其他治疗。另一个实施方案提供了这样的方法，其中所述一种或多种治疗包括以下的一种或多种：放疗、化疗、高剂量化疗加干细胞移植以及单克隆抗体治疗。另一个实施方案提供了这样的方法，其中放疗包括内部和/或外部放疗。另一个实施方案提供了这样的方法，其中化疗包括向受试者施用以下的一种或多种：多柔比星、环磷酰胺、紫杉醇、拉帕替尼、卡培他滨、曲妥珠单抗、贝伐珠单抗、吉西他滨、艾瑞布林或白蛋白结合型紫杉醇。另一个实施方案提供了这样的方法，其中化疗包括向受试者施用一种或多种IGF-1R抑制剂。另一个实施方案提供了这样的方法，其中IGF-1R抑制剂是AEW541。

[0153] 一个实施方案提供了一种治疗有需要的患者的非小细胞肺癌的方法，包括口服施用厄洛替尼和恩替司他，其中恩替司他施用于禁食患者。另一个实施方案提供了治疗非小细胞肺癌的方法，其中施用后恩替司他Tmax少于1小时。另一个实施方案提供了治疗非小细胞肺癌的方法，其中施用后恩替司他Tmax少于90分钟。另一个实施方案提供了治疗非小细

胞肺癌的方法，其中施用后恩替司他Tmax少于2小时。另一个实施方案提供了治疗非小细胞肺癌的方法，其中施用后恩替司他Tmax为30分钟至2小时。另一个实施方案提供了治疗非小细胞肺癌的方法，其中口服施用恩替司他后，恩替司他Cmax为至少150ng/mL。另一个实施方案提供了治疗非小细胞肺癌的方法，其中口服施用恩替司他后，恩替司他Cmax为至少125ng/mL。另一个实施方案提供了治疗非小细胞肺癌的方法，其中口服施用恩替司他后，恩替司他Cmax为至少100ng/mL。另一个实施方案提供了治疗非小细胞肺癌的方法，其中口服施用恩替司他后恩替司他Cmax为至少80ng/mL。另一个实施方案提供了治疗非小细胞肺癌的方法，其中口服施用恩替司他后，恩替司他Cmax为至少50ng/mL。另一个实施方案提供了治疗非小细胞肺癌的方法，其中施用约10mg的恩替司他。另一个实施方案提供了治疗非小细胞肺癌的方法，其中施用约1mg至约20mg的恩替司他。另一个实施方案提供了治疗非小细胞肺癌的方法，其中每14天施用一次恩替司他。另一个实施方案提供了治疗非小细胞肺癌的方法，其中施用恩替司他一个月。另一个实施方案提供了治疗非小细胞肺癌的方法，其中所述患者在施用恩替司他前2小时内不进食。另一个实施方案提供了治疗非小细胞肺癌的方法，其中所述患者在施用恩替司他前1小时内不进食。另一个实施方案提供了治疗非小细胞肺癌的方法，其中所述患者在施用恩替司他后1小时内不进食。另一个实施方案提供了治疗非小细胞肺癌的方法，其中所述患者在施用恩替司他后30分钟内不进食。另一个实施方案提供了治疗非小细胞肺癌的方法，其中在与恩替司他施用当日不同的时间施用厄洛替尼。另一个实施方案提供了治疗非小细胞肺癌的方法，其中将厄洛替尼每日一次施用于所述禁食患者。另一个实施方案提供了治疗非小细胞肺癌的方法，其中所述患者在施用厄洛替尼前2小时内不进食。另一个实施方案提供了治疗非小细胞肺癌的方法，其中所述患者在施用厄洛替尼后1小时内不进食。另一个实施方案提供了治疗非小细胞肺癌的方法，其中施用约150mg的厄洛替尼。

口服制剂

[0154] 含有本文所述活性药物成分的口服制剂可包括任何常规使用的口服形式，包括片剂、胶囊、丸剂、含片、锭剂(lozenges)、香锭剂(pastilles)、扁囊剂、微丸、含药口香糖、颗粒、整装散剂、泡腾或非泡腾粉末或颗粒、溶液剂、乳剂、混悬剂、溶液剂、薄片剂(wafers)、分散型胶囊(sprinkles)、酏剂、糖浆剂、口腔含服形式(buccal forms)和口服液体。胶囊可含有活性化合物和惰性填充剂和/或稀释剂的混合物，所述惰性填充剂和/或稀释剂如药学可接受的淀粉(例如，玉米淀粉、马铃薯淀粉或木薯淀粉)、糖类、人工甜味剂、粉状纤维素如结晶纤维素和微晶纤维素，面粉、明胶、树胶等。有用的片剂制剂可通过常规压片、湿法制粒或干法制粒方法并利用药学可接受的稀释剂、粘合剂、润滑剂、崩解剂、表面改性剂(包括表面活性剂)、助悬或稳定剂(包括但不限于硬脂酸镁、硬脂酸、滑石、月桂基硫酸钠、微晶纤维素、羧甲基纤维素钙、聚乙烯吡咯烷酮、明胶、海藻酸、阿拉伯胶、黄原胶、枸橼酸钠、硅酸盐复合物、碳酸钙、甘油、糊精、蔗糖、山梨糖醇、磷酸二钙、硫酸钙、乳糖、高岭土、甘露醇、氯化钠、滑石、干淀粉和糖粉)。在一些实施方案中，表面改性剂包括非离子表面改性剂和阴离子表面改性剂。例如，表面改性剂包括但不限于，泊洛沙姆188、苯扎氯铵、硬脂酸钙、十八醇十六醇混合物、聚西托醇乳化蜡、脱水山梨糖醇酯、胶体二氧化硅、磷酸盐、十二烷基硫酸钠、硅酸镁铝和三乙醇胺。本文的口服制剂可利用标准延迟释放制剂或延时释放制剂改变所述活性化合物的吸收。所述口服制剂也可由含活性成分的水或果汁组成，其根据需要可含有

适当的增溶剂或乳化剂。

口服给药

[0155] 如本文所述，本文所述的联合疗法可同时给予或可以交错方案给予，在化疗过程中在不同于芳香酶抑制剂的时间给予恩替司他。给予所述两种化合物之间的时间差异的范围可为若干分钟、小时、天、周或更长时间。因此，术语组合并不必然指在同一时间给药或以单一剂量给药，而是指每一组分均在需要的治疗期中给药。这些药剂也可通过不同的途径给予。通常对于化疗方案，化疗疗程可在数周之后重复并可能依循给予所述两种化合物相同的时间表，或者可能基于患者应答而修改。

[0156] 在其他实施方案中，本文所提供的药物组合物可以以用于口服给药的固体、半固体或液体剂型提供。如本文所用，口服给药也包括口腔、舌和舌下给药。适宜的口服剂型包括但不限于，片剂、胶囊、烷基、含片、锭剂(lozenges)、香锭剂(pastilles)、扁囊剂、微丸、含药口香糖、颗粒、整装散剂、泡腾或非泡腾粉末或颗粒、溶液剂、乳剂、混悬剂、溶液剂、薄片剂(wafers)、分散型胶囊(sprinkles)、酏剂和糖浆剂。除活性成分外，所述药物组合物可包含一种或多种药学可接受的载体或赋形剂，其包括但不限于，粘合剂、填充剂、稀释剂、崩解剂、润湿剂、润滑剂、助流剂、着色剂、染料泳移抑制剂、甜味剂和矫味剂。

[0157] 粘合剂或制粒机赋予片剂粘着性以确保在压片后片剂扔保持完整。适宜的粘合剂或制粒机包括但不限于，淀粉，如玉米淀粉、马铃薯淀粉和预胶化淀粉(例如，STARCH1500)；明胶；糖类，如蔗糖、葡萄糖、右旋糖、糖蜜和乳糖；天然和合成胶，如阿拉伯胶、海藻酸、海藻酸盐、爱尔兰藓提取物、Panwar胶、印度胶、isabgol husks胶浆、羧甲基纤维素、甲基纤维素、聚乙烯吡咯烷酮(PVP)、硅酸镁铝、落叶松阿拉伯半乳糖、西黄蓍胶粉和古尔胶；纤维素类，如乙基纤维素、醋酸纤维素、羧甲基纤维素钙、羧甲基纤维素钠、甲基纤维素、羟基乙基纤维素(HEC)、羟基丙基纤维素(HPC)、羟基丙基甲基纤维素(HPMC)；微晶纤维素，如AVICEL-PH-101、AVICEL-PH-103、AVICELRC-581、AVICEL-PH-105(FMC Corp.,Marcus Hook,PA)；和其混合物。适宜的填充剂包括但不限于，滑石、碳酸钙、微晶纤维素、纤维素粉末、葡萄糖结合剂、高岭土、甘露醇、硅酸、山梨糖醇、淀粉、预胶化淀粉和其混合物。本文所提供的药物组合物中，可存在约50重量％至约99重量％的粘合剂或填充剂。

[0158] 适宜的稀释剂包括但不限于，磷酸二钙、硫酸钙、乳糖、山梨糖醇、蔗糖、肌醇、纤维素、高岭土、甘露醇、氯化钠、干淀粉和糖粉。一些稀释剂，如甘露醇、乳糖、山梨糖醇、蔗糖和肌醇，当以足够量存在时，可赋予一些压缩片剂特性从而允许其在口中通过咀嚼崩解。这类压缩片剂可用作咀嚼片。

[0159] 适宜的崩解剂包括但不限于，琼脂；膨润土；纤维素，如甲基纤维素和羧甲基纤维素；木材制品；天然海绵；阳离子交换树脂；海藻酸；树胶，如古尔胶和硅酸镁铝HV；柑橘渣；交联纤维素，如交联羧甲基纤维素；交联聚合物，如交联聚维酮；交联淀粉；碳酸钙；微晶纤维素，如羟乙酸淀粉钠；波拉克林钾；淀粉，如玉米淀粉、马铃薯淀粉、木薯淀粉和预胶化淀粉；粘土；aligns；和其混合物。本文所提供的药物组合物中崩解剂的量随制剂类型而变化，并且对本领域的普通技术人员而言是容易辨别的。本文所提供的药物组合物可包含约0.5重量％至约15重量％或约1重量％至约5重量％的崩解剂。

[0160] 适宜的润滑剂包括但不限于，硬脂酸钙；硬脂酸镁；矿物油；轻质矿物油；甘油；山梨糖醇；甘露醇；二醇类，如山嵛酸甘油酯(glycerol behenate)和聚乙二醇(PEG)；硬脂酸；

月桂基硫酸钠;滑石;氢化植物油,包括花生油、棉籽油、葵花籽油、芝麻油、橄榄油、玉米油和大豆油;硬脂酸锌;油酸乙酯;月桂酸乙酯;琼脂;淀粉;石松子;二氧化硅或硅胶,如AEROSIL® 200(W.R.Grace Co.,Baltimore,MD)和CAB-O-SIL®(Cabot Co.of Boston,MA);及其混合物。本文所提供的药物组合物可包含约0.1重量%至约5重量%的润滑剂。

[0161] 适宜的助流剂包括胶体二氧化硅,CAB-O-SIL®(Cabot Co.of Boston,MA)和无石棉滑石。着色剂包括任何批准的、经检验的、水溶性FD&C染料,和悬浮于水合氧化铝上的水不溶性FD&C染料,和色淀以及其混合物。色淀是通过将水溶性染料吸收进入重金属水合氧化物,其得到不溶形式的染料。矫味剂包括从植物如水果提取的天然香料,和产生令人愉悦味觉的合成的化合物混合物如薄荷和水杨酸甲酯。甜味剂包括蔗糖、乳糖、甘露醇、糖浆、甘油和人工甜味剂如糖精和阿斯巴甜。适宜的乳化剂包括明胶、阿拉伯胶、黄蓍胶、膨润土和表面活性剂如聚氧乙烯失水山梨醇单油酸酯(TWEEN® 20)、聚氧乙烯失水山梨醇单油酸酯80(TWEEN®80)和三乙醇胺油酸酯。助悬和分散剂包括羧甲基纤维素钠、果胶、黄蓍胶、硅酸镁铝、阿拉伯胶、羧甲基纤维素钠、羟基丙基甲基纤维素和聚乙烯吡咯烷酮。防腐剂包括甘油、尼泊金甲酯和尼泊金丙酯、苯甲酸、苯甲酸钠和醇。润湿剂包括丙二醇单硬脂酸酯、失水山梨糖醇单油酸酯、二乙二醇单月桂酸酯和聚氧乙烯月桂基醚。溶剂包括甘油、山梨糖醇、乙醇和糖浆。乳剂中利用的非水性液体的实例包括矿物油和棉籽油。有机酸包括柠檬酸和酒石酸。二氧化碳源包括碳酸氢钠和碳酸钠。

[0162] 应当理解很多载体和赋形剂可起若干作用,即使在同一制剂中。

[0163] 在其他实施方案中,本文所提供的药物组合物可以压缩片、模印片、咀嚼锭、速溶片、多重压缩片或肠溶衣片、糖衣片或薄膜衣片提供。肠溶衣片是被可抵抗胃酸作用但溶解或崩解于肠中的物质包被,从而保护活性成分远离胃的酸性环境的压缩片。肠溶衣包括但不限于脂肪酸、脂肪、水杨酸苯酯、蜡类、虫胶、氨合虫胶和醋酸纤维素酞酸酯。糖衣片是包裹有糖衣的压缩片,其可有利地掩盖令人不快的味道或气味并保护片剂避免氧化。薄膜衣片是覆盖有水溶性材料的薄层或薄膜的压缩片。薄膜衣片包括但不限于羟基乙基纤维素、羧甲基纤维素钠、聚乙二醇4000和醋酸纤维素酞酸酯。薄膜衣赋予了与糖衣相同的共性。多重压缩片是通过多于一个压缩循环制得的压缩片,其包括分层片剂和压制包衣片或干包衣片。

[0164] 片剂剂型可从粉状、结晶或颗粒形式的活性成分,单独或与一种或多种本文所述的载体或赋形剂(包括粘合剂、崩解剂、控释聚合物、润滑剂、稀释剂和/或着色剂)组合制得。调味和甜味剂在咀嚼片剂和锭剂的形成中尤其有用。

[0165] 本文所提供的药物组合物可以软胶囊或硬胶囊提供,其可从明胶、甲基纤维素、淀粉或海藻酸钙制得。所述硬明胶胶囊也称为干填充胶囊(DFC),由两部分组成,一部分塞入另一部分中;因此完全包封了活性成分。软弹性胶囊(SEC)是软的、球形壳,例如明胶壳,其通过加入甘油、山梨糖醇或类似的多元醇塑化。软明胶壳可包含防腐剂以预防微生物生长。合适的防腐剂为如本文描述的那些,包括尼泊金甲酯和尼泊金丙酯,以及山梨酸。本文提供的液体、半固体和固体剂型可包裹在胶囊中。合适的液体和半固体剂型包括碳酸丙烯酯、植

物油或甘油三酯的溶液和混悬剂。包含这样溶液的胶囊可如美国专利4,328,245、4,409,239和4,410,545中描述的制备。所述胶囊也可如本领域技术人员已知的进行包被，以改善或维持活性成分的溶出度。

[0166] 在其他实施方案中，本文提供的药物组合物可以以液体和半固体剂型提供，其包括乳剂、溶液、混悬剂、酏剂和糖浆剂。乳剂为两相系统，其中一种液体以小球形式完全分散在另一种液体中，其可以是水包油型或油包水型。乳剂可包括药学上可接受的非水性液体或溶剂、乳化剂和防腐剂。混悬剂可包括药学上可接受的助悬剂和防腐剂。水性醇溶液可包括药学上可接受的醛，例如低级烷基醛的二(低级烷基)缩醛(术语“低级”意指烷基具有1至6个碳原子)，如乙醛二乙基缩醛；和具有一个或多个羟基的水可混溶性溶剂，例如丙二醇和乙醇。酏剂是透明的、甜味的水醇溶液。糖浆剂是糖例如蔗糖的浓缩水溶液，并且还可包含防腐剂。对于液体剂型，例如，在聚乙二醇中的溶液可用足量的药学上可接受的液体载体例如水稀释，从而方便地测量以用于给药。

[0167] 其他有用的液体和半固体剂型包括但不限于，包含本文所提供的活性成分和二烷基化单亚烷基二醇或多亚烷基二醇的那些剂型，所述单亚烷基二醇或多亚烷基二醇包括1,2-二甲氧基甲烷、二乙二醇二甲醚、三乙二醇二甲醚、四乙二醇二甲醚、聚乙二醇-350-二甲醚、聚乙二醇-550-二甲醚、聚乙二醇-750-二甲醚，其中350、550和750是指聚乙二醇的近似平均分子量。这些制剂可进一步包含一种或多种抗氧化剂，例如丁羟甲苯(BHT)、丁羟茴醚(BHA)、没食子酸丙酯、维生素E、氢醌、羟基香豆素、乙醇胺、卵磷脂、脑磷脂、抗坏血酸、苹果酸、山梨糖醇、磷酸、亚硫酸氢盐、焦亚硫酸钠、硫代二丙酸及其酯和二硫代氨基甲酸酯。

[0168] 本文提供的用于口服给药的药物组合物还可以以脂质体、胶束、微球或纳米系统提供。可以如美国专利6,350,458中的描述制备胶束剂型。

[0169] 在其他实施方案中，本文所提供的药物组合物可以以非泡腾或泡腾颗粒剂和粉剂提供以复溶成液体剂型。在非泡腾颗粒剂或粉剂中使用的药学上可接受的载体和赋形剂可以包括稀释剂、甜味剂和润湿剂。在泡腾颗粒剂或粉剂中使用的药学上可接受的载体和赋形剂可以包括有机酸和二氧化碳源。

[0170] 在所有上述剂型中可使用着色剂和矫味剂。

[0171] 本文提供的药物组合物可配制成即释或改良释放剂型，包括延释、缓释、脉冲释放、控释、靶向释放和程序化释放形式。

[0172] 在其他实施方案中，本文所提供的药物组合物可与不会损害所需治疗作用的其他活性成分共同配制，或者与补充所需作用的物质共同配制。

实施例

实施例1在患有局部复发或转移性ER+乳腺癌的绝经后女性以及患有进行性非小细胞肺癌的男性和女性中评价食物对恩替司他的药代动力学的影响的1期随机化开放标签研究

方案

[0173] 标题：在患有局部复发或转移性ER+乳腺癌的绝经后女性以及患有进行性非小细胞肺癌的男性和女性中评价食物对恩替司他的药代动力学的影响的1期研究

研究阶段：1期

适应证：乳腺癌；非小细胞肺癌

主要目标：

• 为了在患有乳腺癌的女性和患有非小细胞肺癌(NSCLC)的男性和女性中评估食物对恩替司他的药代动力学的影响。

次要目标：

• 安全性:为了评估如通过不良事件、实验室参数和心电评价测量的恩替司他与依西美坦或厄洛替尼组合的安全性和耐受性

探索目标：

• 为了确定食物对通过恩替司他施用诱导的蛋白质赖氨酸乙酰化变化的程度的影响

• 为了评估接受恩替司他的患者的、与恩替司他血浆浓度和治疗持续时间相关的乙酰化程度。

• 为了确定依西美坦或厄洛替尼的添加是否达到通过恩替司他施用诱导的乙酰化变化的程度

研究设计：

[0174] 这是恩替司他的I期、随机化、开放标签、两阶段、两组交叉研究。将患者以1:1的比例随机分组,以在第1周期第1天(C1D1)在有或无食物的情况下接受恩替司他10mg。被随机分组为在C1D1在有食物的情况下接受恩替司他的患者将在第1周期第15天(C1D15)在无食物的情况下接受第二剂量的恩替司他10mg。相似地,被随机分组为在C1D1在无食物的情况下接受恩替司他的患者将在C1D15在有食物的情况下接受第二剂量的恩替司他10mg。随机分组将通过性别分层。研究中的每个周期将持续28天。将在给药前获得血液样品,并且将在每次给药后取得系列血液样品以评价药代动力学。此外,将抽取血液样品用于评价恩替司他乙酰化。

[0175] 对于第2周期和所有随后的周期,所有患者都将继续在每个周期的第1天和第15天接受10mg的恩替司他。患有乳腺癌的那些患者还将从第2周期第1天开始每日一次(qd)口服(po)接受依西美坦25mg。患有NSCLC的那些患者还将从第2周期第1天开始每日一次口服接受150mg厄洛替尼。

[0176] 将采用标准临床和实验室评价,在筛选时以及在研究登记期间的预先规定的时间对患者进行评价。还将在每2个周期后评价患者的肿瘤响应。将通过CT、MRI或其他合适的放射学研究来评价肿瘤发展。患者将继续接受其合适的研究治疗周期直至肿瘤发展或不良事件发生,如由研究者确定的该肿瘤发展或不良事件的发生使得必需停止治疗。

终点：

主要药代动力学终点

• Cmax,最大血浆浓度

• Tmax,最大血浆浓度的时间

• AUClast,从时间零到最后的可测量浓度的在血浆浓度-时间曲线下的面积

[0177] AUCinf,通过下述的AUClast+Clast/λz从时间零外推至无穷的在血浆浓度-时间曲线下的面积

• λz,终点消除速率常数

次要药效学终点

• 如通过外周血单核细胞测量的蛋白质赖氨酸乙酰化中相对于基线的变化

[0178] 安全性终点

• 治疗紧急的不良事件、严重不良事件、导致研究药物永久停止的不良事件、以及在研究药物最后给药的30天内发生的死亡的发生率

• 实验室、生命体征和心电图结果从基线的变化

[0179] 样品大小:将有多达28名患者(约14名患有乳腺癌的患者和14名患有NSCLC的患者,其中最少4名男性患者)参加,以确保24名患者(每个治疗组约12名)完成研究治疗的第1周期。

受试者合格标准概述:该研究将招收在诊断时患有组织学或细胞学上确认的雌激素受体阳性(ER+)乳腺癌的绝经后女性,其疾病已经发展到研究者确定该患者为接受依西美坦的候选者的程度。此外,将招收患有细胞学或组织学上确认的IIIb或IV期NSCLC的成年人,其为厄洛替尼治疗的合格候选人。所有患者必须至少18岁,且东部肿瘤协作组(ECOG)状态为0或1。

研究的产品:

[0180] 恩替司他是合成小分子,其具有分子式C21H20N4O3和376.41的分子量。恩替司他被分类为抗肿瘤剂,具体通过促进核小体组蛋白的高度乙酰化而起到组蛋白脱乙酰酶抑制剂的作用。恩替司他是口服可生物利用的,并且将作为含有5.0mg活性成分的黄色包衣片剂提供。

研究治疗:

一个周期将被确定为28天的研究治疗。

所有患者:仅第1周期

• A组:在测试禁食情况下第1天和在测试进食情况下第15天口服恩替司他10mg

• B组:在测试进食情况下第1天和在测试禁食情况下第15天口服恩替司他10mg

仅乳腺癌患者:第2周期和所有后续周期:

• 将从第2周期第1天开始每日一次施用依西美坦25mg

• 在第1天和第15天早餐后至少2小时口服施用10mg剂量的恩替司他,随后为至少1小时的禁食

仅NSCLC患者:第2周期和所有后续周期

• 将从第2周期第1天开始每日一次施用厄洛替尼150mg

• 在第1天和第15天早餐后至少2小时口服施用10mg剂量的恩替司他,随后为至少1小时的禁食

[0181] 药代动力学评价,所有患者(仅研究第1周期的第1和15天)

根据患者的随机分组,使患者施用以下两种治疗中的一种:在测试进食情况下10mg恩替司他或在测试禁食情况下10mg恩替司他。所有治疗均将以单剂量和240mL水一起给予。在给药前最多2小时内根据需要在所有治疗组中水将是被允许的,然后直到给药后2小时水是被限制的(除了在进食治疗组中早餐期间饮用的液体)。将获取血液以用于确定在以下时间的恩替司他浓度:给药前(在给药的60分钟内),以及随后在给药后0.25、0.5、1、1.5、2、3、4、6、8、10、12、24、72、120、168、240和336小时。将在给药前-60和-45分钟以及随后在与PK血液样品相同的时间点一式三份获取ECG。将在第1周期的第1天和第15天从给药前到给药后12小时使用Holter监视器。

[0182] 药效动力学评价,所有患者

研究第1周期的第1天和第15天

将获取血液以用于确定在以下时间的恩替司他乙酰化:给药前(在给药的60分钟内),以及随后在给药后12、24、168和336小时。

研究第3周期的第1天

将获取血液以用于确定在第3周期第1天给药前的蛋白质乙酰化。

研究评价的结束

将获取血液以用于确定在最终研究访视时的蛋白质乙酰化。

研究持续时间

患者将继续接受方案治疗,直至遭受进行性疾病或不可接受的或无法耐受的毒性。

统计注意事项:

[0183] 将使用针对区间假设的Schuirmann双向单侧检验程序来比较进食和禁食状态的平均生物利用度的差异。将通过基于AUC_{last}和C_{max}的恩替司他暴露的程度来确定平均生物利用度的差异。

[0184] 将测试以下一组假设以用于根据对数转换后的数据比较进食状态与禁食状态:

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

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

其中μ_{Fed}和μ_{Fasted}分别表示进食和禁食情况下的总体平均AUC_{last}、AUC_{inf}或C_{max}。总共24名可评估的患者(每个治疗组12名)将被要求以90%的概率和5%的I型错误率来检测上述提到的进食和禁食情况之间平均生物利用度的差异。患者间变异系数(CV)被假定为22%。进食与禁食情况之间的真实平均比值被假定为1.0。

[0185] 如果根据通过随机分组确定的条件(即,进食或禁食)患者在每个治疗期中均接受恩替司他,则他们将被认为是PK分析可评估的。患者还必须有足够的来自各个治疗期的血浆浓度-时间数据,以提供PK参数(例如,Cmax、AUClast)的有意义的评价。

[0186] 单个患者浓度-时间数据将被列出并以线性和对数标尺图形展示。浓度-时间数据将以表格和图形形式(线性和对数标尺)描述性地总结。使用非房室方法估算的PK参数将采用WinNonlin 5.1版本或更高版本进行计算。这类估算值将被列出并以表格和图形形式描述性地总结。

[0187] 对于选定的PK参数(例如,Cmax、AUClast),将采用线性混合效应ANOVA模型来进行进食与禁食情况之间的比较。该模型将包括治疗情况(进食、禁食)、阶段和作为固定影响因素的组、以及作为随机影响因素的组内患者。生物等效性的评价将基于经典(最短)置信区间方法,该方法在操作上相当于区间假设的Schuirmann双向单侧检验程序。将使用来自ANOVA模型的估算值来计算进食和禁食情况下的真实平均AUC的比值的90%置信限。如果比值(回转换的)的90%置信区间完全包含在80%到125%的等同限内,则将推断出平均生物利用度的生物等效性。

[0188] 将采用描述性统计和图形显示来总结进食和禁食情况下达到恩替司他的最大观察血浆浓度的时间(Tmax)。将计算进食与禁食情况之间Tmax的单个患者差异;将提供中位数差的对称非参数置信区间。将采用Wilcoxon符号秩检验(Wilcoxon's signed rank test)来进行进食与禁食情况之间Tmax的推理比较。

[0189] 蛋白质赖氨酸乙酰化的变化(该研究的主要药效动力学参数)将以与如上文针对

PK分析所述的相同或相似的方式进行分析。也可以进行经由乙酰化的PK联合分析。

[0190] 对所有接受至少一个剂量的恩替司他的患者进行安全性数据分析。分析结果将由针对临床和实验室参数以及不良事件的数据汇总组成。除非另外说明，将通过初步诊断来进行安全性分析。将通过与单一研究治疗的关系并通过严重性等级来总结有一个或多个不良事件的患者的数目和百分比。将采用NCI-CTCAE(4.0版本)来确定严重性等级。将采用医疗监管活动术语词典(Medical Dictionary for Regulatory Activities Terminology，MedDRA)来编码不良事件。将采用描述性统计通过相对于基线的改变以及临床显著异常的数据列表来总结实验室参数。将采用描述性统计通过相对于基线值的变化来总结生命体征和ECG数据。

结果

[0191] 本文中示出了食物效应研究的结果。图1示出了在禁食或进食情况下施用10mg恩替司他后的平均浓度时间图。表1中示出了药代动力学参数的总结。

[0192] 10mg恩替司他与食物共施用导致药物吸收的滞后和Tmax的延迟(在禁食情况下中值tmax＝0.76小时；在进食情况下中值tmax＝11小时)。观察到最大药物浓度的显著降低(Cmax降低71％)。当恩替司他与高脂肪膳食一起施用时，通过AUClast和AUCinf估算的总体暴露减少了约15-17％。恩替司他的平均消除半衰期被估算为在禁食情况下140小时和在进食情况下178小时。在进食组的半衰期估算中存在高的变异度(％CV＝70％)，这可能归因于小的样本大小。在进食组中少数个体确实具有显著延长的t1/2值。t1/2的中值表明两个组是相当的。

[0193] 结论：当恩替司他与高脂肪膳食共施用时食物效应对于恩替司他是显而易见的，导致延迟的tmax以及降低的Cmax和AUC。

实施例2

[0194] 通过施用恩替司他和非甾体芳香酶抑制剂、依西美坦治疗患有局部复发或转移性雌激素受体阳性乳腺癌的绝经后女性的方法。

[0195] 该研究的目的是评估恩替司他与依西美坦组合在治疗晚期乳腺癌中的安全性和有效性。

[0196] 主要结果测定是比较依西美坦单用和依西美坦加恩替司他的有效性，其通过自随机化日期测量的无进展生存期(PFS)的持续时间来测定。

[0197] 次要结果测定是比较客观应答率(ORR)和临床受益率(CBR)，

并评估恩替司他和依西美坦组合的安全性和耐受性，其通过不良事件和实验室安全性参数测量。

[0198] 研究设计

组（Arm）	指定干预
1：实验 每日依西美坦（阿诺新） 25 mg 加每周一次恩替司他 5 mg PO 干预： •药物：恩替司他 •药物：依西美坦	药物：恩替司他 在没有食物的情况下每周一次 5 mg PO 恩尼诺特片剂 药物：依西美坦 25 mg 依西美坦 PO QD 其他名称：阿诺新（Aromasin）
2：安慰剂对照组 每日依西美坦（阿诺新） 25mg 加每周一次安慰剂 PO 干预：药物：依西美坦	药物：依西美坦 依西美坦 25 mg PO QD 其他名称：阿诺新（Aromasin）

合格标准：

符合研究的年龄：≥18岁

符合研究的性别:女性

接受健康志愿者:否

纳入标准：

• 绝经后女性患者

• 组织学上或细胞学上确认的ER+乳腺癌

• 之前经AI治疗后复发或进展的

• 转移性疾病必须是可测量的

• 在入选前,在非靶病变处接受姑息放射的患者必须在治疗结束后有两周的清洗期。

• 患者之前可接受一种化疗作为一线疗法的部分,只要其在之前AI治疗开始前接受的即可。

• ECOG行为状况:0至1

• 实验室参数:a)血红蛋白≥9.0g/dL;血小板≥100.0x10<9>/L;ANC≥1.5x 10<9>/L,未使用造血生长因子;b)对于该机构,肌酐低于正常值上限的2.5倍;c)对于该机构,AST和ALT低于正常值上限的2.5倍

• 能够理解并作出书面知情同意书且顺从研究规程

排除标准：

• 辅助治疗设置中的患者经非甾体AI治疗后少于12个月复发

• 转移性疾病患者经最近AI治疗少于3个月后疾病进展

• 快速进展,威胁生命的转移

• 任何对可测量病变的姑息性放疗

• 此前经SNDX-275或任何其他HDAC抑制剂(包括丙戊酸)治疗

• 对苯甲酰胺类或研究药物的非活性组分过敏

• 对依西美坦的任何活性或非活性成分有过敏史

• 任何妨碍足够的研究治疗依从性的并发医疗病症

• 患者目前正入选(或在研究药物给药前30天内结束)另一项试验药物研究

• 患者目前正接受丙戊酸、Zolinza(伏立诺他)或任何其他的HDAC抑制剂或DNA甲基转移酶抑制剂或任何全身抗癌治疗(除醋酸亮丙瑞林外)治疗

实施例3

[0199] 一种通过施用厄洛替尼和恩替司他的联合治疗患有非小细胞肺癌的患者的方法，该患者在使用厄洛替尼后进展。

[0200] 主要结果测定：

疾病控制率(完全响应、部分响应或持续至少3个月的稳定疾病)

[0201] 次要结果测定：

在第2个月时的无进展存活率

在第4个月时的无进展存活率

[0202] 研究设计

组	指定干预
1：实验 “厄洛替尼响应性”患者是在对厄洛替尼完全或部分响应，或者持续至少 3 个月的稳定疾病期后进展的患者。 干预： 药物：恩替司他 药物：厄洛替尼	药物：恩替司他 在无食物情况下，在 28-天周期的第 1 和 15 天施用恩替司他(10 mg 固定剂量 PO Q2W)，持续最多 6 个周期 药物：厄洛替尼 厄洛替尼(150 mg PO QD)，持续最多六 (6) 个 28-天周期
2：实验 “厄洛替尼非响应性”患者是在采用厄洛替尼治疗期间 (即，在至少 1 个厄洛替尼治疗完全周期	药物：恩替司他 在无食物情况下，在 28-天周期的第 1 和 15 天施用恩

之后）立即进展的，或具有客观反应或持续少于3个月的稳定疾病期的患者。 干预： 药物：恩替司他 药物：厄洛替尼	替司他(10 mg 固定剂量 PO Q2W)，持续最多 6 个周期 药物：厄洛替尼 厄洛替尼(150 mg PO QD)，持续最多六（6）个 28-天周期

合格标准

符合研究的年龄:18岁及以上

符合研究的性别:两者

接受健康志愿者:否

入选标准：

●具有细胞学或组织学上确认的IIIb期(胸腔积液)或IV期NSCLC

●基于至少2次扫描(最后一次是在研究登记的4周内并可以充当用于患者筛选入该研究的基线扫描),疾病对于厄洛替尼治疗是进展的(对治疗没有反应或随后在客观反应后复发)

●从与大多数最近的癌症治疗相关的任何毒性(不大于CTCAE尺度1级毒性或达到先前的基线条件)中恢复

●通过常规CT扫描至少1个可测量的损伤≥20mm,或通过螺旋CT扫描至少1个可测量的损伤≥10mm

●ECOG体能分数为0、1或2,且至少3个月的预期寿命

●石蜡包埋的肿瘤样本可用于相关研究

●超过18岁的男性或女性

●血红蛋白≥9.0g/dL;血小板≥75x 10^9/L;ANC≥1.0x 10^9/L,未使用造血生长因子

●凝血试验在正常范围内

●对于该机构,胆红素和肌酐低于正常值上限的2倍

●对于该机构,AST和ALT低于正常值上限的3倍

●对于该机构,钾、镁和磷在正常范围内(补充是被允许的)

●在研究期间以及恩替司他最后给药后3个月内愿意采用公认且有效的避孕方法(男性和女性均适用)

●在任何研究特异性过程(包括特殊的筛选测试)之前,患者或法律上可接受的代表已同意书面知情同意书

排除标准：

●先前进行干细胞移植

●有症状的CNS累及

●先前用HDAC抑制剂治疗

●并存的抗癌治疗,用于非靶向研究病变的放疗除外

●目前正服用在违禁药物名单上的药物

●在登记前28天内全身性化疗或用试验药治疗

●目前使用丙戊酸

●未治疗或不稳定的脑转移,或在研究药物施用的4周内针对该状况服用类固醇

●目前活跃的继发恶性肿瘤,或在过去5年内的任何恶性肿瘤,治愈的基底或鳞状细胞皮肤癌、宫颈原位癌或浅表性膀胱癌除外

●无法吞咽口服药物或胃肠吸收不良状况

●需要IV抗生素、抗病毒药或抗真菌药的不受控制的感染,已知的HIV感染,或活跃的乙型肝炎或丙型肝炎感染

●定义为在ECG上临床显著发现的异常心功能(多病灶PVC,与心肌梗死或急性缺血一致的ST-T波变化,QTc大于500毫秒),心动过速或在MUGA扫描上左心室射血分数低于40%

●在登记的3个月内另一种严重或不受控制的医学状况如高血压、糖尿病或抑制的免疫系统

●已知对苯甲酰胺过敏

●病态肥胖

●目前怀孕或哺乳的女性

●目前参与(或在28天内完成)另一个试验药研究的患者

●不能用于研究中(on-study)或随访评价的患者

●患者具有使患者参与研究时风险增加或损害患者作出书面知情同意书的能力和/或损害其遵从研究程序及要求的能力的任何种类的医学、精神或行为障碍

表1:在禁食和进食情况下施用10mg恩替司他后药代动力学参数的总结

阶段		T1/2 (hr)	Tlag (hr)	Tmax (hr)	Cmax (ng/mL)	AUClast (ng*hr/mL)	AUCinf (ng*hr/mL)	Cmax 比	AUClast 比	AUCinf 比
禁食	N	13	16	16	16	16	13	15	15	9
	平均值	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
	中值	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
进食	N	10	16	16	16	16	10			
	平均值	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			
	中值	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比＝进食的Cmax/禁食的Cmax

AUClast比＝进食的AUClast/禁食的AUClast

AUCinf比＝进食的AUCinf/禁食的AUCinf

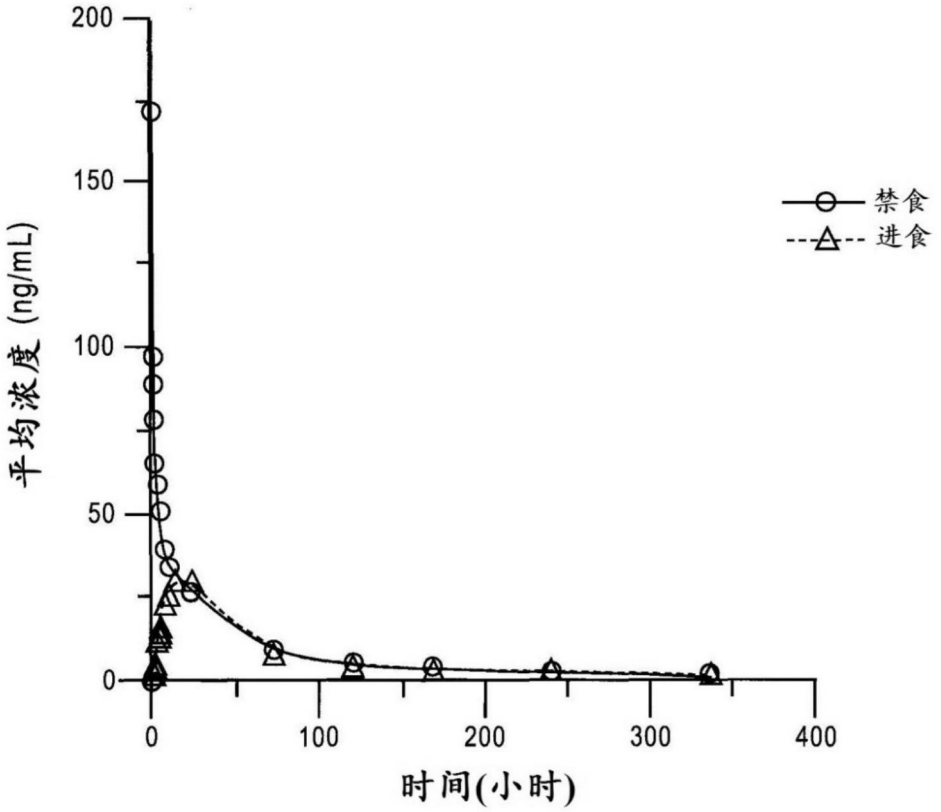

200
150
100
50
0
平均浓度 (ng/mL)
0
100
200
300
400
时间(小时)
禁食
进食

图1(a)

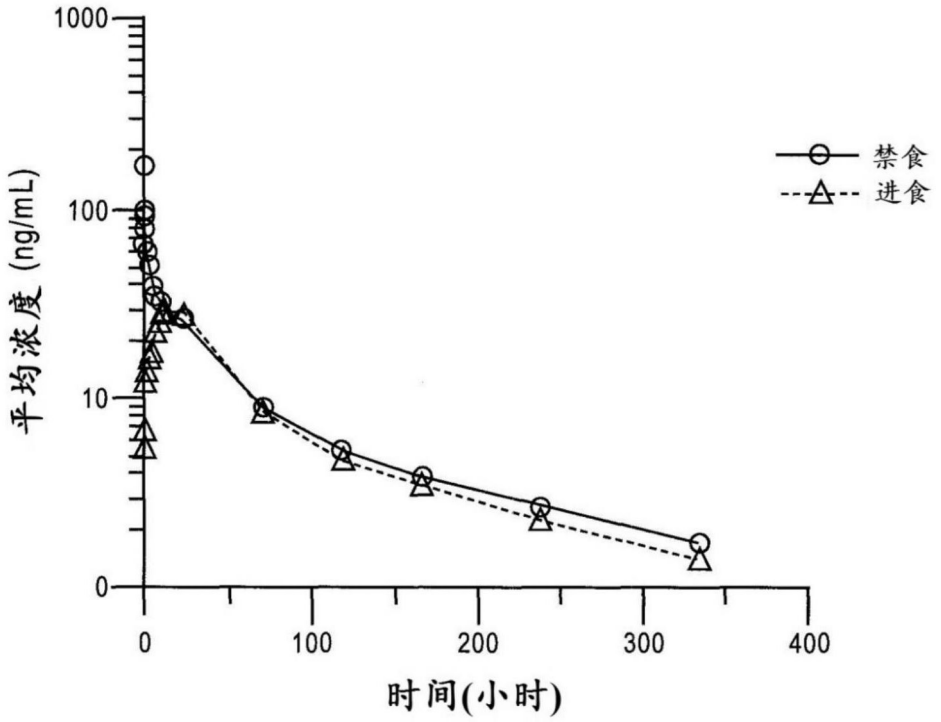

1000
100
10
0
平均浓度 (ng/mL)
0
100
200
300
400
时间(小时)
禁食
进食

图1(b)