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(54) **ADMINISTRATION OF NEDD8-ACTIVATING ENZYME INHIBITOR AND CHEMOTHERAPEUTIC AGENTS**

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(57) **ABSTRACT**

Disclosed are methods for the treatment of various solid tumors in patients in need of such treatment. The methods comprise administering to such a patient an NEDD8-activating enzyme (NAE) inhibitor such as (1S,2S,4R)-4-4-(1S)-2,3-dihydro 1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]-pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate (MLN4924) or a pharmaceutically acceptable salt in combination with one or more chemotherapeutic agents. Also disclosed are medicaments for use in the treatment of various solid tumors.

FIGURE 1

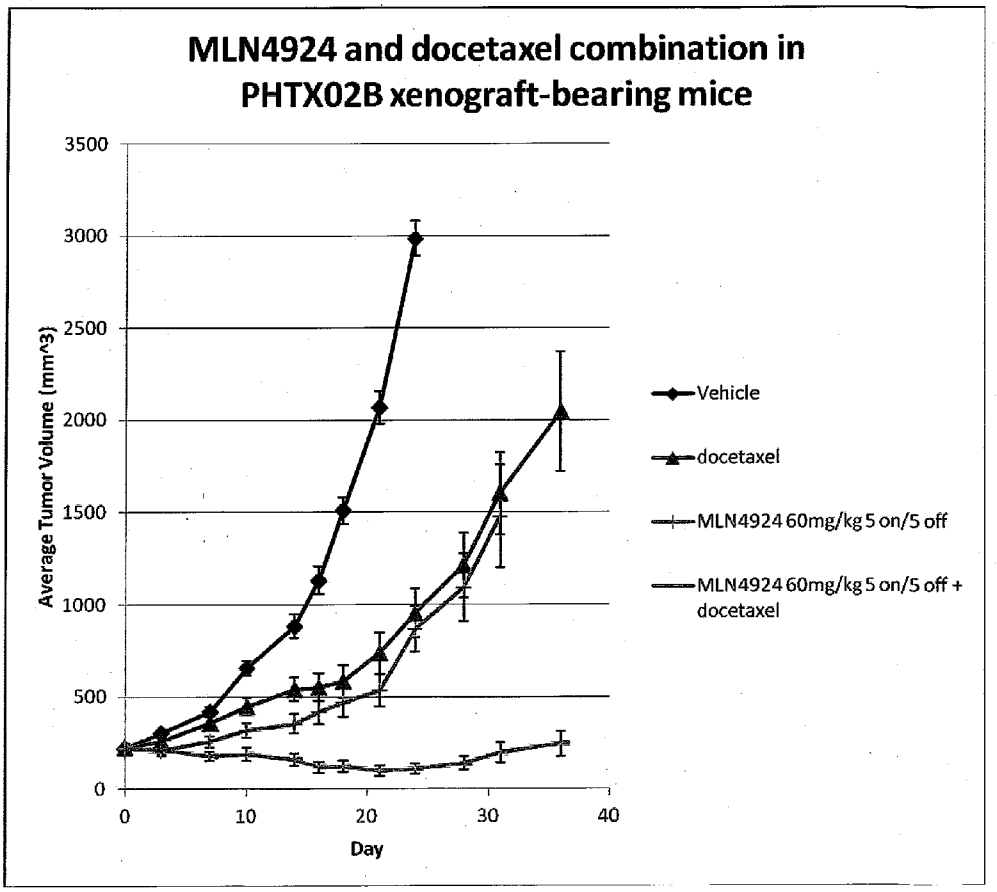

MLN4924 and docetaxel combination in PHTX02B xenograft-bearing mice
Average Tumor Volume (mm^3)
3500
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
Day
Vehicle
docetaxel
MLN4924 60mg/kg 5 on/5 off
MLN4924 60mg/kg 5 on/5 off + docetaxel

FIGURE 2

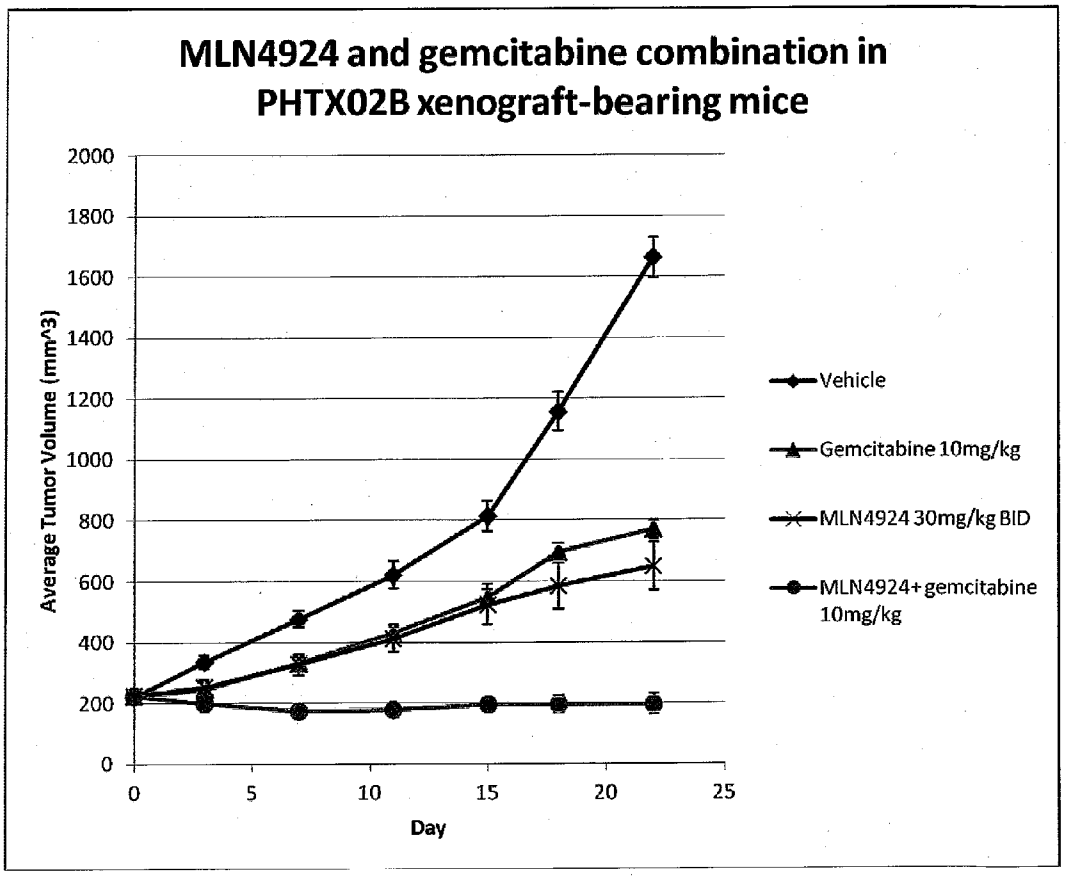

MLN4924 and gemcitabine combination in PHTX02B xenograft-bearing mice
Average Tumor Volume (mm^3)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
5
10
15
20
25
Day
Vehicle
Gemcitabine 10mg/kg
MLN4924 30mg/kg BID
MLN4924+gemcitabine 10mg/kg

FIGURE 3

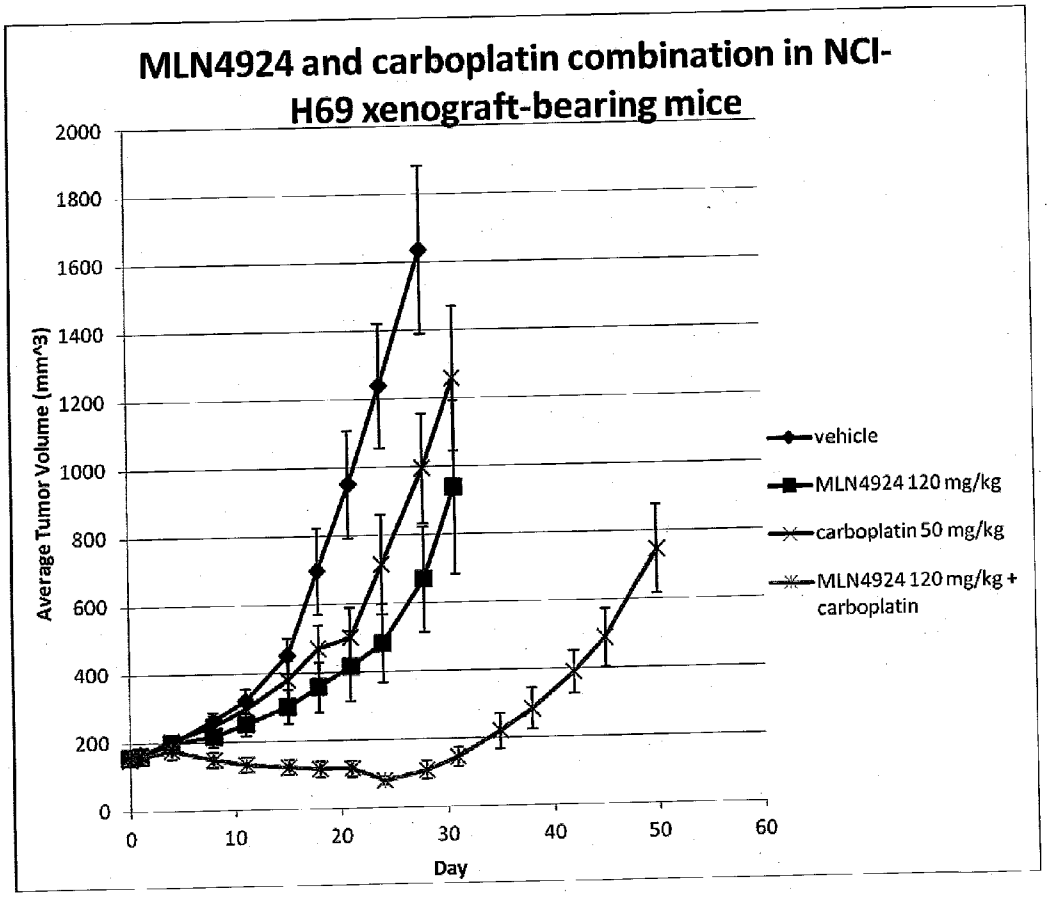
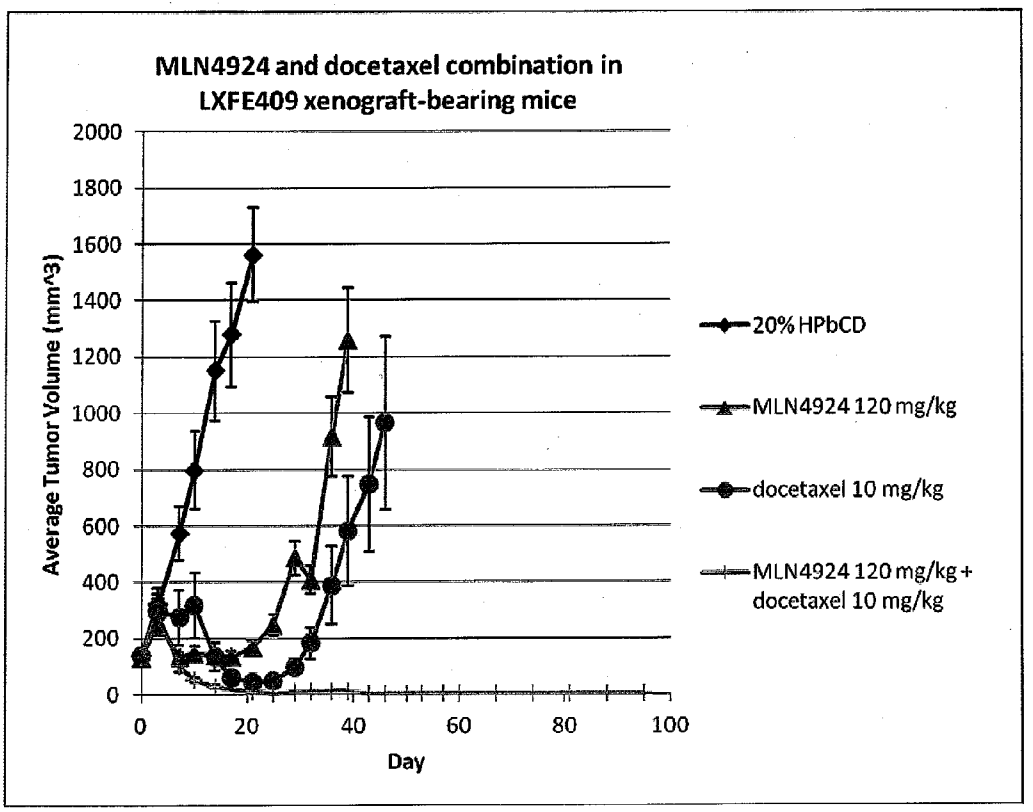

MLN4924 and carboplatin combination in NCI-H69 xenograft-bearing mice
Average Tumor Volume (mm^3)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60
Day
vehicle
MLN4924 120 mg/kg
carboplatin 50 mg/kg
MLN4924 120 mg/kg + carboplatin

FIGURE 4


MLN4924 and docetaxel combination in LXFE409 xenograft-bearing mice
Average Tumor Volume (mm^3)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
20
40
60
80
100
Day
20% HPbCD
MLN4924 120 mg/kg
docetaxel 10 mg/kg
MLN4924 120 mg/kg + docetaxel 10 mg/kg

ADMINISTRATION OF NEDD8-ACTIVATING ENZYME INHIBITOR AND CHEMOTHERAPEUTIC AGENTS

PRIORITY CLAIM

[0001] This application claims priority to U.S. Provisional Application No. 61/822,994 filed on May 14, 2013, to U.S. Provisional Application No. 61/874,393 filed on Sep. 6, 2013 and to U.S. Provisional Application No. 61/891,943 filed on Oct. 17, 2013. The entire contents of each of the foregoing applications are incorporated herein by reference.

FIELD

[0002] This present disclosure relates to oncology and to methods for the treatment of cancer. In particular, the present disclosure provides methods for treatment of various solid tumors by administering a NEDD8-activating enzyme (NAE) inhibitor in combination with one or more chemotherapeutic agents.

BACKGROUND

[0003] Cancer is the second most common cause of death in the U.S. and accounts for one of every eight deaths worldwide. The National Cancer Institute estimates that approximately 13.7 million Americans with a history of cancer were alive on Jan. 1, 2012. Some of these individuals were cancer free, while others still had evidence of cancer and may have been undergoing treatment. About 1,660,290 new cancer cases are expected to be diagnosed in the US in 2013. In 2013, about 580,350 Americans are expected to die of cancer, almost 1,600 people per day. Although medical advances have improved cancer survival rates, there remains a continuing need for new and more effective treatment. Currently available treatments for solid tumors include neoadjuvant chemotherapy and/or radiation therapy and adjuvant chemotherapy and/or radiation therapy following surgical removal or resection. In addition, there are a number of newer targeted therapies that are also used in the treatment of various solid tumors.

[0004] Inhibition of NEDD8-activating enzyme (NAE) has been shown to induce cancer cell death and inhibit the growth of tumors in xenograft models. See, e.g., T. A. Soucy et al., *Nature,* 2009, 458, 732-737; T. A. Soucy et al., *Clin. Cancer Res.,* 2009, 15 (12), 3912-3916; and J. E. Brownell et al., *Mol. Cell.,* 2010, 37 (1), 102-111. Reports of Phase I clinical studies of an NAE inhibitor include R. T. Swords et al., *Blood,* 2010, 115, 3796-3800; J. S. Kauh et al., *J. Clin. Oncol.,* 2011, 29, abstract 3013; and S. Bhatia et al., *J. Clin. Oncol.,* 2011, 29, abstract 8529. Inhibitors of NAE are described in U.S. patent application Ser. No. 11/346,469 (Publ. No. 2006/0189636, U.S. Pat. No. 7,951,810), Ser. No. 11/700,614 (Publ. No. 2007/0191293) and Ser. No. 11/890,338 (Publ. No. 2008/0051404, U.S. Pat. No. 8,008,307).

[0005] New combinations of therapeutic agents that provide a beneficial effect in the treatment of solid tumors are desirable in order to prolong patient's lives while maintaining a high quality of life. Further, new combinations may provide an increased benefit as compared to each of the agents alone. This is especially true in the case where the solid tumors may be resistant or refractory to currently available therapeutic regimens.

SUMMARY

[0006] In one aspect, the present disclosure relates to methods of treating cancer comprising administering an NAE inhibitor and one or more chemotherapeutic agents in combination to a subject in need of such treatment.

[0007] In one aspect, the present disclosure relates to a kit comprising a medicament for use in treating cancer in a subject in need of such treatment. The kit comprises a medicament comprising an NAE inhibitor, and instructions for administering the NAE inhibitor and the one or more chemotherapeutic agents; or the kit comprises a medicament comprising the one or more chemotherapeutic agents, and instructions for administering the one or more chemotherapeutic agents and a NAE inhibitor. The kit can contain both a medicament comprising an NAE inhibitor and a medicament comprising one or more chemotherapeutic agents, and instructions for administering the NAE inhibitor and the one or more chemotherapeutic agents.

[0008] In one aspect, the present disclosure relates to a medicament for use in treating cancer in a subject in need of such treatment. The medicament comprises an NAE inhibitor and one or more chemotherapeutic agents.

BRIEF DESCRIPTION OF THE FIGURES

[0009] FIG. **1** shows a plot of tumor volume as a function of time in a PHTX02B xenograft model following administration of MLN4924 and docetaxel to mice.

[0010] FIG. **2** shows a plot of tumor volume as a function of time in a PHTX02B xenograft model following administration of MLN4924 and gemcitabine to mice.

[0011] FIG. **3** shows a plot of tumor volume as a function of time in a NCI-H69 xenograft model following administration of MLN4924 and carboplatin to mice.

[0012] FIG. **4** shows a plot of tumor volume as a function of time in a LXFE409 xenograft model following administration of MLN4924 and docetaxel to mice.

DESCRIPTION

Definitions and Abbreviations

[0013] AUC area under the plasma concentration versus time curve
BSA body surface area
CR complete response
MTD maximum tolerated dose
NAE Nedd8-activating enzyme
Nedd8 neural precursor cell expressed, developmentally down-regulated 8
PR partial response
QD once daily
SCLC small cell lung cancer

[0014] As used herein, the term "cancer" refers to a cellular disorder characterized by uncontrolled or dysregulated cell proliferation, decreased cellular differentiation, inappropriate ability to invade surrounding tissue, and/or ability to establish new growth at ectopic sites. The term "cancer" includes solid tumors and hematological tumors. The term "cancer" encompasses diseases of skin, tissues, organs, bone, cartilage, blood, and vessels. The term "cancer" further encompasses primary and metastatic cancers.

[0015] As used herein, "clinically effective amount" means an amount of a therapeutic substance that is sufficient upon appropriate administration to a patient (a) to cause a detect-

able decrease in the severity of the disorder or disease state being treated; (b) to ameliorate or alleviate the patient's symptoms of the disease or disorder; or (c) to slow or prevent advancement of, or otherwise stabilize or prolong stabilization of, the disorder or disease state being treated (e.g., prevent additional tumor growth of a cancer).

[0016] When more than one therapeutic substance is being administered, the "clinically effective total amount" means that the sum of the individual amounts of each therapeutic substance meets the definition of "clinically effective amount" even if the individual amounts of any number of the individual therapeutic substances would not. For example, if 10 mg of A were not a clinically effective amount, and 20 mg of B were not a clinically effective amount, but the administration of 10 mg A+20 mg B resulted in at least one of the results enumerated for the definition of "clinically effective amount", then the sum of 10 mg A+20 mg B would be considered a "clinically effective total amount".

[0017] In any form or composition, the administered dose (s) or the clinically effective (total) amount can be expressed as amount(s) of therapeutic substance(s) per patient BSA, e.g., as mg/m^2.

[0018] As used herein, "patient" means a human being diagnosed with, exhibiting symptoms of or otherwise believed to be afflicted with a disease, disorder or condition.

[0019] As used herein, "body surface area" (BSA) is calculated using a standard nomogram, e.g.,

$$BSA(m^2) = \sqrt{\frac{Ht(cm) \times Wt(kg)}{3600}} \quad \text{or} \quad BSA = \sqrt{\frac{Ht(in) \times Wt(lb)}{3131}}$$

[0020] As used herein, dosing for carboplatin is based upon an estimate of the GFR (glomerular filtration rate) and the desired level of drug exposure, according to the area under the curve of concentration X time (AUC, mg/mL×min), rather than the more common dosing calculation based upon the body surface area (mg/m^2). For a desired target AUC (which typically varies between 5 and 7 mg/mL×min) and the estimated GFR, the dose of carboplatin is then calculated by use of the Calvert formula:

Total carboplatin dose, mg=Target AUC×(estimated creatinine clearance+25).

Because of potential changes in weight or renal function, this calculation should be repeated prior to each administered course of carboplatin.

[0021] The estimation of the GFR is based upon a calculation of creatinine clearance according to the Cockcroft-Gault Equation (Cockcroft D W, Gault M H. Prediction of creatinine clearance from serum creatinine. Nephron. 1976; 16(1): 31-41):

For males:

Creatinine Clearance=(140−age [years]×weight [kg])/72×(serum creatinine [mg/dL])

For females:

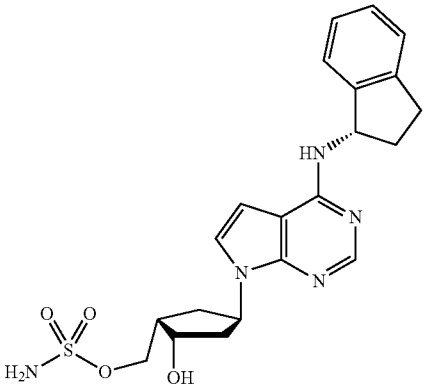
Creatinine Clearance=0.85(140−age [years]×weight [kg])/72×(serum creatinine [mg/dL]).

[0022] As used herein, the illustrative terms "include", "such as", "for example" and the like (and variations thereof; e.g., "includes" and "including", "examples"), unless otherwise specified, are intended to be non-limiting. That is, unless explicitly stated otherwise, such terms are intended to imply "but not limited to", e.g., "including" means including but not limited to.

DETAILED DESCRIPTION

[0023] In some embodiments, the present disclosure relates to a method of treating solid tumors in a patient by administering to a patient a combination of MLN4924 and one or more chemotherapeutic agents, wherein the chemotherapeutic agent is: (i) a taxane; (ii) a platin; or (iii) gemcitabine.

[0024] The compound ((1S,2S,4R)-4-(4-((1S)-2,3-dihydro-1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]-pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate:



MLN4924

also known as MLN4924, is an inhibitor of NEDD8-activating enzyme (NAE). See, e.g., T. A. Soucy et al., *Nature,* 2009, 458, 732-737; T. A. Soucy et al., *Clin. Cancer Res.,* 2009, 15 (12), 3912-3916; and J. E. Brownell et al., *Mol. Cell.,* 2010, 37 (1), 102-111, each of which is hereby incorporated by reference herein in its entirety. MLN4924, pharmaceutical compositions of MLN4924, processes for its synthesis, and polymorphic forms have been described previously. See, e.g., U.S. patent application Ser. No. 11/700,614 (Publ. No. 2007/0191293), Ser. No. 12/221,399 (Publ. No. 2009/0036678) and Ser. No. 12/779,331 (Publ. No. 2011/0021544), each of which is hereby incorporated by reference herein in its entirety. If there is any discrepancy between any of these documents and the present specification, the present specification controls.

[0025] In another aspect, the present disclosure relates to the use of MLN4924 or a pharmaceutically acceptable salt in combination with one or more chemotherapeutic agents, wherein the chemotherapeutic agent is: (i) a taxane; (ii) a platin; or (iii) gemcitabine for the treatment of solid tumors.

[0026] In another aspect, the present disclosure relates to the use of MLN4924 or a pharmaceutically acceptable salt in combination with one or more chemotherapeutic agents, wherein the chemotherapeutic agent is: (i) a taxane; (ii) a platin; or (iii) gemcitabine in the manufacture of a medicament for use in treating solid tumors.

[0027] In another aspect, the present disclosure relates to the use of MLN4924 or a pharmaceutically acceptable salt in the manufacture of a medicament for treating solid tumors, wherein the MLN4924 or a pharmaceutically acceptable salt thereof is administered with one or more chemotherapeutic

agents, wherein the chemotherapeutic agent is: (i) a taxane; (ii) a platin; or (iii) gemcitabine.

[0028] In another aspect, the present disclosure relates to a kit for treating solid tumors comprising at least one medicament comprising at least one dose of MLN4924 or a pharmaceutically acceptable salt thereof, and at least one medicament comprising at least one dose of one or more of: (i) a platin; (ii) a taxane; or (iii) gemcitabine or a pharmaceutically acceptable salt thereof, said kit for treating solid tumors further comprising dosing instructions for administering the medicaments for treatment of the subject in recognized need thereof.

[0029] MLN4924 or a pharmaceutically acceptable salt thereof can be administered in combination with the one or more chemotherapeutic agents in a single dosage form or as a separate dosage forms. In one embodiment, when administered as a separate dosage form, the one or more chemotherapeutic agents can be administered prior to, at the same time as, or following administration of MLN4924. In some embodiments, when administered as a separate dosage form, one or more doses of MLN4924 or a pharmaceutically acceptable salt thereof, may be administered prior to the one or more chemotherapeutic agents. In some embodiments, the one or more therapeutic agents is administered prior to the administration of MLN4924 or a pharmaceutically acceptable salt thereof. As used herein, the administration in "combination" of MLN4924 and a chemotherapeutic agent refers not only to simultaneous or sequential administration of the two agents, but also to the administration of both compounds during a single treatment cycle, as understood by one skilled in the art. When MLN4924 or a pharmaceutically acceptable salt thereof is administered in combination with the one or more chemotherapeutic agents a clinically effective total amount is administered.

[0030] In some embodiments, MLN4924 or a pharmaceutically acceptable salt is administered intravenously (IV). In some embodiments, the one or more chemotherapeutic agents is administered intravenously (IV).

[0031] In some embodiments, the one or more chemotherapeutic agent is one chemotherapeutic agent. In some embodiments, the one or more chemotherapeutic agents is two chemotherapeutic agents. In some embodiments, the one or more chemotherapeutic agents is three chemotherapeutic agents.

[0032] In some embodiments, the chemotherapeutic agent is a platinum containing compound ("platin"). Platinum containing compounds include agents such as cisplatin, carboplatin, oxaliplatin, satraplatin, picoplatin, nedaplatin and triplatin. Platinum containing chemotherapeutic agents cause crosslinking of DNA as monoadduct, interstrand crosslinks, intrastrand crosslinks or DNA protein crosslinks. The resulting crosslinking inhibits DNA repair and/or DNA synthesis in cancer cells. These agents are sometimes described as being alkylating-like agents despite the fact that they do not have an alkyl group. Cisplatin was the first platinum containing compound to be discovered and was first approved by the U.S. Food and Drug Administration in 1978. Carboplatin was introduced in the 1980s and has been demonstrated to have lower side-effects than cisplatin in ovarian cancer and lung cancer (Hartmann and Lipp, *Exper. Opin. Pharmacother.* 2003, 4(6) 889-901).

[0033] In some embodiments, the platin is cisplatin, carboplatin, oxaliplatin, satraplatin, picoplatin, nedaplatin or triplatin. In some embodiments, the platin is nedaplatin, cisplatin, carboplatin or oxaliplatin. In some embodiments, the platin is cisplatin, carboplatin or oxaliplatin. In some embodiments, the platin is cisplatin. In some embodiments, the platin is carboplatin. In some embodiments, the platin is cisplatin or carboplatin.

[0034] In some embodiments, the chemotherapeutic agent is gemcitabine or a pharmaceutically acceptable salt. Gemcitabine is a nucleoside analog that works by disrupting DNA replication. Gemcitabine is approved for treatment by the U.S. Food and Drug Administration for the treatment of several cancers including pancreatic cancer, non-small cell lung cancer and breast cancer. Gemcitabine can be in the form of a pharmaceutically acceptable salt, such as an acid addition salt. In some embodiments, the acid addition salt of gemcitabine is gemcitabine hydrochloride. One form of gemcitabine is currently marketed as GEMZAR® (Eli Lilly and Company).

[0035] In some embodiments, the chemotherapeutic agent is a taxane. Taxanes are diterpenes produced by the plants of the genus *Taxus* (yew trees). Taxanes were first discovered and isolated from this natural source but are mostly now produced by synthetic or semi-synthetic methods. The principle mechanism by which taxanes exert their effect is the disruption of microtubule function during cell division, thereby preventing effective growth and division of cancer cells.

[0036] Taxane agents include paclitaxel and docetaxel. Paclitaxel was originally isolated from the bark of the Pacific yew tree and was subsequently produced in a semi-synthetic manner Paclitaxel was first approved by the U.S. Food and Drug Administration in 1992. Docetaxel is also derived semi-synthetically from the needles of the yew tree. Docetaxel is approved by the U.S. Food and Drug Administration for the treatment of advanced breast, lung, and ovarian cancer. An alternative formulation of paclitaxel where the paclitaxel is bound to albumin nano-particles, known as nab-paclitaxel [marketed as Abraxane (Celgene Corporation)] is also approved by the U.S. Food and Drug Administration for certain types of metastatic breast cancer. In some embodiments, the taxane is paclitaxel, docetaxel or nab-paclitaxel. In some embodiments, the taxane is paclitaxel or docetaxel. In some embodiments, the taxane is paclitaxel. In some embodiments, the taxane is docetaxel.

[0037] In some embodiments, the cancer is a solid tumor. In some embodiments, the solid tumor is breast cancer, colon cancer, lung cancer, pancreatic cancer, bladder cancer, esophageal cancer, or head and neck cancer. In some embodiments, the solid tumor is breast cancer, colon cancer, lung cancer, pancreatic cancer, bladder cancer, esophageal cancer, head and neck cancer or cholangiocarcinoma. In some embodiments, the solid tumor is breast cancer, colon cancer, lung cancer or pancreatic cancer. In some embodiment, the solid tumor is colon cancer, lung cancer or pancreatic cancer. In some embodiments, the solid tumor is lung cancer or pancreatic cancer. In some embodiments, the solid tumor is lung cancer, head and neck cancer or cholangiocarcinoma. In some embodiments, the solid tumor is lung cancer or head and neck cancer.

[0038] In some embodiments, the solid tumor is lung cancer. Lung cancer includes different sub-types such as small cell lung cancer (SCLC); non-small cell lung cancer (NSCLC) including squamous NSCLC; bronchioloalveolar carcinoma (BAC); and adenocarcinoma. In some embodiments, the solid tumor is small cell lung cancer. In some

embodiments, the solid tumor is non-small cell lung cancer. In some embodiments, the solid tumor is squamous non-small cell lung cancer.

[0039] In some embodiments, the solid tumor is breast cancer. Breast cancer includes different sub-types such as luminal A, luminal B, triple-negative (basal-like) and HER-2 type. In some embodiments, the solid tumor is triple-negative breast cancer.

[0040] In some embodiments, the solid tumor is esophageal cancer. Esophageal cancer includes sub-types of adenocarcinoma and squamous. In some embodiments, the solid tumor is squamous esophageal cancer.

[0041] In some embodiments, the solid tumor is head and neck cancer. Head and neck cancer are those that arise, in the head and neck region and the cancer may be found in areas such as nasal cavities, sinuses, lips, mouth, salivary glands, pharynx or larynx. 90% of head and neck cancers are squamous cell carcinomas (SCCHN), which originate from the mucosal lining (epithelium) of these regions. In some embodiments, the solid tumor is squamous head and neck cancer.

[0042] In some embodiments, the solid tumor is colon cancer. In some embodiments, the solid tumor is pancreatic cancer. In some embodiments, the solid tumor is biliary tract cancers which include cholangiocarcinoma, pancreatic cancer, gallbladder cancer, and cancer of the ampulla of Vater. In some embodiments, the solid tumor is cholangiocarcinoma.

[0043] In some embodiments, the solid tumor is bladder cancer. Bladder cancer includes both non-invasive and invasive sub-types. In some embodiments, the solid tumor is invasive bladder cancer.

[0044] In some embodiments, the method of treatment further comprises the use of radiotherapy. The radiotherapy may be administered prior to the administration of the combination or after the administration of the combination.

[0045] In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on each of days 1, 3, and 5 of a 21 day schedule is less than or equal to 50 mg/m^2. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on each of days 1, 3, and 5 of a 21 day schedule is 50 mg/m^2. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on each of days 1, 3, and 5 of a 21 day schedule is 37 mg/m^2. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on each of days 1, 3, and 5 of a 21 day schedule is 25 mg/m^2. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on each of days 1, 3, and 5 of a 21 day schedule is 15 mg/m^2. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on each of days 1, 3, and 5 of a 21 day schedule is 20 mg/m^2. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on each of days 1, 3, and 5 of a 21 day schedule is about 10 mg/m^2 to about 30 mg/m^2.

[0046] In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 8, and 15 of a 28 day schedule. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on each of days 1, 8, and 15 of a 28 day, schedule is less than or equal to 100 mg/m^2. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on each of days 1, 8, and 15 of a 28 day schedule is 100 mg/m^2. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on each of days 1, 8, and 15 of a 28 day schedule is 75 mg/m^2. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on each of days 1, 8, and 15 of a 28 day schedule is 50 mg/m^2. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on each of days 1, 8, and 15 of a 28 day schedule is 25 mg/m^2. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on each of days 1, 8, and 15 of a 28 day schedule is 20 mg/m^2. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on each of days 1, 8, and 15 of a 28 day schedule is 15 mg/m^2. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on each of days 1, 8, and 15 of a 28 day schedule is about 15 mg/m^2 to about 40 mg/m^2.

[0047] In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on day 1 of a 21 day schedule. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on day 1 of a 21 day schedule is less than or equal to 50 mg/m^2. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on day 1 of a 21 day schedule is less than or equal to 25 mg/m^2. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on day 1 of a 21 day schedule is 20 mg/m^2. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on day 1 of a 21 day schedule is less than or equal to 15 mg/m^2. In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on day 1 of a 28 day schedule. In some embodiments, the amount of MLN4924 or a pharmaceutically acceptable salt thereof that is administered on day 1 of a 28 day schedule is less than or equal to 100 mg/m^2.

[0048] In some embodiments, the one or more chemotherapeutic agents is administered on day 1 of a 21 day schedule. In some embodiments, a taxane is administered on day 1 of a 21 day schedule. In some embodiments, docetaxel is administered on day 1 of a 21 day schedule. In some embodiments, the amount of docetaxel that is administered on day 1 of a 21 day schedule is 75 mg/m^2. In some embodiments, the amount of docetaxel that is administered on day 1 of a 21 day schedule is about 50 mg/m^2 to about 100 mg/m^2. In some embodiments, paclitaxel is administered of day 1 of a 21 day schedule. In some embodiments, the amount of paclitaxel that is administered on day 1 of a 21 day schedule is 200 mg/m^2. In some embodiments, the amount of paclitaxel that is administered on day 1 of a 21 day schedule is 175 mg/m^2. In some embodiments, the amount of paclitaxel that is administered on day 1 of a 21 day schedule is 135 mg/m^2. In some embodiments, the amount of paclitaxel that is administered on day 1 of a 21 day schedule is about 135 mg/m^2 to about 200 mg/m^2.

[0049] In some embodiments, a platin is administered on day 1 of a 21 day schedule. In some embodiments, carboplatin is administered on day 1 of a 21 day schedule. In some

embodiments, the amount of carboplatin that is administered on day 1 of a 21 day schedule is AUC 6 (calculated as per the Calvert calculation above). In some embodiments, the amount of carboplatin that is administered on day 1 of 21 day schedule is AUC 5.

[0050] In some embodiments, cisplatin is administered on day 1 of a 21 day schedule. In some embodiments, the total amount of cisplatin administered is less than or equal to 100 mg/m^2. In some embodiments, the amount of cisplatin administered on day 1 of a 21 day schedule is about 75 mg/m^2 to about 100 mg/m^2. In some embodiments, the amount of cisplatin administered on day 1 of a 21 day schedule is about 50 mg/m^2 to about 70 mg/m^2. In some embodiments, cisplatin is administered on each of days 1, 2, and 3 of a 21 day schedule. In some embodiments, cisplatin is administered on each of days 1, 3, and 5 of a 21 day schedule. In some embodiments, the total amount of cisplatin administered is less than or equal to 100 mg/m^2. In some embodiments, the amount of cisplatin that is administered on each of days 1, 2, and 3 of a 21 day schedule is 25 mg/m^2. In some embodiments, the amount of cisplatin that is administered on each of days 1, 3, and 5 of a 21 day schedule is 25 mg/m^2.

[0051] In some embodiments, the one or more chemotherapeutic agents is administered on each of days 1, 8, and 15 of a 28 day schedule. In some embodiments, gemcitabine is administered on each of days 1, 8, and 15 of a 28 day schedule. In some embodiments, the amount of gemicitabine that is administered on each of days 1, 8, and 15 of a 28 day schedule is 1000 mg/m^2. In some embodiments, the amount of gemicitabine that is administered on each of days 1, 8, and 15 of a 28 day schedule is 800 mg/m^2.

[0052] In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule, and the one or more chemotherapeutic agent is administered on day 1 of a 21 day schedule. In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule, and one chemotherapeutic agent is administered on day 1 of a 21 day schedule. In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule, and two chemotherapeutic agents are administered on day 1 of a 21 day schedule.

[0053] In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule, and a taxane is administered on day 1 of a 21 day schedule. In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule, and docetaxel is administered on day 1 of a 21 day schedule. In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule, and paclitaxel is administered on day 1 of a 21 day schedule.

[0054] In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule, and a platin is administered on day 1 of a 21 day schedule. In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule, and carboplatin is administered on day 1 of a 21 day schedule. In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule, and oxaliplatin is administered on day 1 of a 21 day schedule.

[0055] In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule, and cisplatin is administered on day 1 of a 21 day schedule. In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule, and cisplatin is administered on each of days 1, 2, and 3 of a 21 day schedule. In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule, and cisplatin is administered on each of days 1, 3, and 5 of a 21 day schedule.

[0056] In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule, and a taxane and a platin are administered on day 1 of a 21 day schedule. In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule, and paclitaxel and carboplatin are administered on day 1 of a 21 day schedule. In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule, and paclitaxel and cisplatin are administered on day 1 of a 21 day schedule. In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule, and docetaxel and carboplatin are administered on day 1 of a 21 day schedule. In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule, and docetaxel and cisplatin are administered on day 1 of a 21 day schedule.

[0057] In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 8, and 15 of a 28 day schedule and the one or more chemotherapeutic agent is administered on each of days 1, 8, and 15 of a 28 day schedule. In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 8, and 15 of a 28 day schedule, and one chemotherapeutic agent is administered on each of days 1, 8, and 15 of a 28 day schedule. In some embodiments, MLN4924 or a pharmaceutically acceptable salt thereof is administered on each of days 1, 8, and 15 of a 28 day schedule, and gemcitabine is administered on each of days 1, 8, and 15 of a 28 day schedule.

[0058] In some embodiments, wherein the solid tumor is colon cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and a taxane. In some embodiments, wherein the solid tumor is colon cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and docetaxel. In some embodiments, wherein the solid tumor is colon cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and paclitaxel.

[0059] In some embodiments, wherein the solid tumor is colon cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and a platin. In some embodiments, wherein the solid tumor is colon cancer, the method comprises administering to a patient in need of such

treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and oxaliplatin.

[0060] In some embodiments, wherein the solid tumor is breast cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and a taxane. In some embodiments, wherein the solid tumor is breast cancer, the method comprises administering to, a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and docetaxel. In some embodiments, wherein the solid tumor is breast cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and paclitaxel.

[0061] In some embodiments, wherein the solid tumor is lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, and a platin. In some embodiments, wherein the solid tumor is lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, and carboplatin. In some embodiments, wherein the solid tumor is lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, and cisplatin. In some embodiments, wherein the solid tumor is lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, and nedaplatin. In some embodiments, wherein the solid tumor is small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, and carboplatin. In some embodiments, wherein the solid tumor is small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, and cisplatin. In some embodiments, wherein the solid tumor is small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, and nedaplatin. In some embodiments, wherein the solid tumor is non-small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, and carboplatin. In some embodiments, wherein the solid tumor is non-small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, and cisplatin. In some embodiments, wherein the solid tumor is non-small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, and nedaplatin. In some embodiments, wherein the solid tumor is squamous non-small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, and carboplatin. In some embodiments, wherein the solid tumor is squamous non-small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, and cisplatin. In some embodiments, wherein the solid tumor is squamous non-small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, and nedaplatin.

[0062] In some embodiments, wherein the solid tumor is lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, a taxane and a platin. In some embodiments, wherein the solid tumor is lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, paclitaxel and carboplatin. In some embodiments, wherein the solid tumor is small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, paclitaxel and carboplatin. In some embodiments, wherein the solid tumor is non-small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, paclitaxel and carboplatin. In some embodiments, wherein the solid tumor is squamous non-small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, paclitaxel and carboplatin.

[0063] In some embodiments, wherein the solid tumor is lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, gemcitabine and a platin. In some embodiments, wherein the solid tumor is lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, gemcitabine and carboplatin. In some embodiments, wherein the solid tumor is small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, gemcitabine and carboplatin. In some embodiments, wherein the solid tumor is non-small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, gemcitabine and carboplatin. In some embodiments, wherein the solid tumor is squamous non-small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, gemcitabine and carboplatin. In some embodiments, wherein the solid tumor is lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, gemcitabine and cisplatin. In some embodiments, wherein the solid tumor is small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, gemcitabine and cisplatin. In some embodiments, wherein the solid tumor is non-small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, gemcitabine and cisplatin. In some embodiments, wherein the solid tumor is squamous non-small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, gemcitabine and cisplatin.

[0064] In some embodiments, wherein the solid tumor is lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and a taxane. In some embodiments, wherein the solid tumor is lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and docetaxel. In some embodiments, wherein the solid tumor is small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and docetaxel. In some embodiments, wherein the solid tumor is non-small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, and docetaxel. In some embodiments, wherein the solid tumor is squamous non-small cell lung cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, and docetaxel.

[0065] In some embodiments, wherein the solid tumor is pancreatic cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and gemcitabine.

[0066] In some embodiments, wherein the solid tumor is invasive bladder cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, gemcitabine and cisplatin. In some embodiments, wherein the solid tumor is invasive bladder cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, gemcitabine and carboplatin.

[0067] In some embodiments, wherein the solid tumor is squamous esophageal cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and a taxane. In some embodiments, wherein the solid tumor is squamous esophageal cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and docetaxel.

[0068] In some embodiments, wherein the solid tumor is head and neck cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and a taxane. In some embodiments, wherein the solid tumor is head and neck cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and docetaxel. In some embodiments, wherein the solid tumor is squamous head and neck cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and docetaxel. In some embodiments, wherein the solid tumor is salivary gland cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, and docetaxel.

[0069] In some embodiments, wherein the solid tumor is cholangiocarcinoma, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and a taxane. In some embodiments, wherein the solid tumor is cholangiocarcinoma, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof and docetaxel.

[0070] In some embodiments, wherein the solid tumor is head and neck cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, a taxane and a platin. In some embodiments, wherein the solid tumor is head and neck cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, paclitaxel and carboplatin. In some embodiments, wherein the solid tumor is squamous head and neck cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, paclitaxel and carboplatin. In some embodiments, wherein the solid tumor is salivary gland cancer, the method comprises administering to a patient in need of such treatment a combination of MLN4924 or a pharmaceutically acceptable salt thereof, paclitaxel and carboplatin.

Therapeutic Substance; Pharmaceutical Compositions.

[0071] Any of therapeutic agents described herein can be in the form of a pharmaceutically acceptable salt. In some embodiments, such salts are derived from inorganic or organic acids or bases. For reviews of suitable salts, see, e.g., Berge et al., *J Pharm. Sci.,* 1977, 66, 1-19 and *Remington: The Science and Practice of Pharmacy*, 20th Ed., A. Gennaro (ed.), Lippincott Williams & Wilkins (2000).

[0072] Examples of suitable acid addition salts include acetate, adipate, alginate, aspartate, benzoate, benzene sulfonate, bisulfate, butyrate, citrate, camphorate, camphor sulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, lucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate and undecanoate.

[0073] Examples of suitable base addition salts include ammonium salts; alkali metal salts, such as sodium and potassium salts; alkaline earth metal salts, such as calcium and magnesium salts; salts with organic bases, such as dicyclohexylamine salts, N-methyl-D-glucamine; and salts with amino acids such as arginine, lysine, and the like.

[0074] For example, Berge lists the following FDA-approved commercially marketed salts: anions acetate, besylate (benzenesulfonate), benzoate, bicarbonate, bitartrate, bromide, calcium edetate (ethylenediaminetetraacetate), camsylate (camphorsulfonate), carbonate, chloride, citrate, dihydrochloride, edetate (ethylenediaminetetraacetate), edisylate (1,2-ethanedisulfonate), estolate (lauryl sulfate), esylate (ethanesulfonate), fumarate, gluceptate (glucoheptonate), gluconate, glutamate, glycollylarsanilate (glycollamidophenylarsonate), hexylresorcinate, hydrabamine (N,N'-di(dehydroabietyl)ethylenediamine), hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isethionate (2-hydroxyethanesulfonate), lactate, lactobionate, malate, maleate, mandelate, mesylate (methanesulfonate), methylbromide, methylnitrate, methylsulfate, mucate, napsylate (2-naphthalenesulfonate), nitrate, pamoate (embonate), pantothenate, phosphate/

diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, sulfate, tannate, tartrate, teoclate (8-chlorotheophyllinate) and triethiodide; organic cations benzathine (N,N'-dibenzylethylenediamine), chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine; and metallic cations aluminum, calcium, lithium, magnesium, potassium, sodium and zinc.

[0075] Berge additionally lists the following non-FDA-approved commercially marketed (outside the United States) salts: anions adipate, alginate, aminosalicylate, anhydromethylenecitrate, arecoline, aspartate, bisulfate, butylbromide, camphorate, digluconate, dihydrobromide, disuccinate, glycerophosphate, hemisulfate, hydrofluoride, hydroiodide, methylenebis(salicylate), napadisylate (1,5-naphthalenedisulfonate), oxalate, pectinate, persulfate, phenylethylbarbiturate, picrate, propionate, thiocyanate, tosylate and undecanoate; organic cations benethamine (N-benzylphenethylamine), clemizole (1-p-chlorobenzyl-2-pyrrolildine-1'-ylmethylbenzimidazole), diethylamine, piperazine and tromethamine (tris(hydroxymethyl)aminomethane); and metallic cations barium and bismuth.

[0076] As used herein, "pharmaceutically acceptable carrier" refers to a material that is compatible with a recipient subject (a human) and is suitable for delivering an active agent to the target site without terminating the activity of the agent. The toxicity or adverse effects, if any, associated with the carrier preferably are commensurate with a reasonable risk/benefit ratio for the intended use of the active agent.

[0077] The pharmaceutical compositions for use in the methods of the present disclosure can be manufactured by methods well known in the art such as conventional granulating, mixing, dissolving, encapsulating, lyophilizing, or emulsifying processes, among others. Compositions can be produced in various forms, including granules, precipitates, or particulates, powders, including freeze dried, rotary dried or spray dried powders, amorphous powders, tablets, capsules, syrup, suppositories, injections, emulsions, elixirs, suspensions or solutions. Formulations can contain stabilizers, pH modifiers, surfactants, solubilizing agents, bioavailability modifiers and combinations of these.

[0078] Pharmaceutically acceptable carriers that can be used in these compositions include ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates or carbonates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

[0079] These pharmaceutical compositions are formulated for pharmaceutical administration to a human being. Such compositions can be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intraperitoneal, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. In some embodiments, the compositions are administered orally, intravenously or subcutaneously. In some embodiments, the compositions are administered orally. In some embodiments, the compositions are administered intravenously. These formulations can be designed to be short-acting, fast-releasing, or long-acting. Furthermore, the compositions can be administered in a local rather than systemic means, such as administration (e.g., by injection) at a tumor site.

[0080] Pharmaceutical formulations can be prepared as liquid suspensions or solutions using a liquid, such as an oil, water, an alcohol, and combinations of these. Solubilizing agents such as cyclodextrins can be included. Pharmaceutically suitable surfactants, suspending agents, or emulsifying agents, can be added for oral or parenteral administration. Suspensions can include oils, such as peanut oil, sesame oil, cottonseed oil, corn oil and olive oil. Suspension preparations can also contain esters of fatty acids such as ethyl oleate, isopropyl myristate, fatty acid glycerides and acetylated fatty acid glycerides. Suspension formulations can include alcohols, such as ethanol, isopropyl alcohol, hexadecyl alcohol, glycerol and propylene glycol; ethers, such as poly(ethyleneglycol); petroleum hydrocarbons such as mineral oil and petrolatum; and water.

[0081] Sterile injectable forms of these pharmaceutical compositions can be aqueous or oleaginous suspensions. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions can also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents which are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as sorbitan alkyl esters, such as Tweens or Spans, and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms can also be used for the purposes of formulation. Compounds can be formulated for parenteral administration by injection such as by bolus injection or continuous infusion. A unit dosage form for injection can be in ampoules or in multi-dose containers.

[0082] These pharmaceutical compositions can be orally administered in any orally acceptable dosage form including capsules, tablets, aqueous suspensions or solutions. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents can also be added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. In the case of tablets for oral use, carriers that are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. Coatings may be used for a variety of purposes, e.g., to mask taste, to

affect the site of dissolution or absorption, or to prolong drug action. Coatings can be applied to a tablet or to granulated particles for use in a capsule.

[0083] Alternatively, these pharmaceutical compositions can be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient which is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

[0084] These pharmaceutical compositions can also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

[0085] Topical application for the lower intestinal tract may be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches can also be used. For topical applications, the pharmaceutical compositions can be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of the present disclosure include mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical compositions can be formulated in a suitable lotion or cream containing the active component(s) suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

[0086] For ophthalmic use, the pharmaceutical compositions can be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with our without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the pharmaceutical compositions can be formulated in an ointment such as petrolatum.

[0087] The pharmaceutical compositions can also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well known in the art of pharmaceutical formulation and can be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

[0088] The methods of the present disclosure are directed to treating diseases, disorders and conditions in which inhibition of NAE enzyme activity is detrimental to survival and/or expansion of diseased cells or tissue (e.g., cells are sensitive to NAE inhibition; inhibition of NAE activity disrupts disease mechanisms; reduction of NAE activity stabilizes protein which are inhibitors of disease mechanisms; reduction of NAE activity results in inhibition of proteins which are activators of disease mechanisms). The diseases, disorders and conditions are also intended to include those which require effective cullin and/or ubiquitination activity, which activity can be regulated by diminishing NAE enzyme activity.

[0089] In some embodiments, the methods of the present disclosure further comprise administering a anti-cancer agent. As used herein, the term "anticancer agent" refers to any agent that is administered to a subject with cancer for purposes of treating the cancer. The administration of the further anti-cancer agent includes administration concurrently or sequentially with the combinations of the present disclosure. Alternatively, the further anti-cancer agent can be combined into one composition with the combinations of the present disclosure which is administered to the patient.

[0090] Non-limiting examples of anti-cancer agents include DNA damaging chemotherapeutic agents such as topoisomerase I inhibitors (e.g., irinotecan, topotecan, camptothecin and analogs or metabolites thereof, and doxorubicin); topoisomerase II inhibitors (e.g., etoposide, teniposide, and daunorubicin); alkylating agents (e.g., melphalan, chlorambucil, busulfan, thiotepa, ifosfamide, carmustine, lomustine, semustine, streptozocin, decarbazine, methotrexate, pemetrexed, mitomycin C, and cyclophosphamide); DNA intercalators; DNA intercalators and free radical generators such as bleomycin; and nucleoside mimetics (e.g., 5-fluorouracil, capecitibine, fludarabine, cytarabine, mercaptopurine, thioguanine, pentostatin, and hydroxyurea). Chemotherapeutic agents that disrupt cell replication include: vincristine, vinblastin, and related analogs; thalidomide, lenalidomide, and related analogs (e.g., CC-5013 and CC-4047); protein tyrosine kinase inhibitors (e.g., imatinib mesylate, erlotonib, crortinib and gefitinib); proteasome inhibitors (e.g., bortezomib); NF-κB inhibitors, including inhibitors of IκB kinase; antibodies which bind to proteins overexpressed in cancers and thereby downregulate cell replication (e.g., trastuzumab, panitumumab, rituximab, cetuximab, and bevacizumab); and other inhibitors of proteins or enzymes known to be upregulated, over-expressed or activated in cancers, the inhibition of which downregulates cell replication.

Kits

[0091] In some embodiments, one or more of the therapeutic agents described herein can be manufactured for inclusion in a kit. A "kit" is any article of manufacture (e.g., a package or container) comprising at least one reagent or chemotherapeutic agent. A kit for use in the methods herein can comprise an NAE inhibitor, such as MLN4924 or a pharmaceutically acceptable salt thereof. In some embodiments, the kit can further include a taxane. In some embodiments, the kit can further include a platin. In some embodiments, the kit can further include gemcitabine. In some embodiments, the kit can further include a taxane and a platin. In some embodiments, the kit can include MLN4924 or a pharmaceutically acceptable salt thereof and docetaxel. In some embodiments, the kit can include MLN4924 or a pharmaceutically acceptable salt thereof and cisplatin. In some embodiments, the kit can include MLN4924 or a pharmaceutically acceptable salt thereof and carboplatin. In some embodiments, the kit can include MLN4924 or a pharmaceutically acceptable salt thereof, carboplatin and paclitaxel.

[0092] In some embodiments, a kit for use in treating lung cancer can include MLN4924 or a pharmaceutically acceptable salt thereof and docetaxel. In some embodiments, a kit for use in treating non-small cell lung cancer can include MLN4924 or a pharmaceutically acceptable salt thereof and docetaxel. In some embodiments, a kit for use in treating squamous non-small cell lung cancer can include MLN4924 or a pharmaceutically acceptable salt thereof and docetaxel. In some embodiments, a kit for use in treating head and neck cancer can include MLN4924 or a pharmaceutically accept-

able salt thereof and docetaxel. In some embodiments, a kit for use in treating salivary gland cancer can include MLN4924 or a pharmaceutically acceptable salt thereof and docetaxel. In some embodiments, a kit for use in treating squamous head and neck cancer can include MLN4924 or a pharmaceutically acceptable salt thereof and docetaxel. In some embodiments, a kit for use in treating cholangiocarcinoma can include MLN4924 or a pharmaceutically acceptable salt thereof and docetaxel.

[0093] In some embodiments, a kit for use in treating lung cancer can include MLN4924 or a pharmaceutically acceptable salt thereof, paclitaxel and carboplatin. In some embodiments, a kit for use in treating non small cell lung cancer can include MLN4924 or a pharmaceutically acceptable salt thereof, paclitaxel and carboplatin. In some embodiments, a kit for use in treating squamous non small cell lung cancer can include MLN4924 or a pharmaceutically acceptable salt thereof, paclitaxel and carboplatin. In some embodiments, a kit for use in treating head and neck cancer can include MLN4924 or a pharmaceutically acceptable salt thereof, paclitaxel and carboplatin. In some embodiments, a kit for use in treating salivary gland cancer can include MLN4924 or a pharmaceutically acceptable salt thereof, paclitaxel and carboplatin. In some embodiments, a kit for use in treating squamous head and neck cancer can include MLN4924 or a pharmaceutically acceptable salt thereof, paclitaxel and carboplatin.

[0094] In some embodiments, a kit comprising MLN4924 or a pharmaceutically acceptable salt thereof and another chemotherapeutic agent can further include another component or reagent. In some embodiments, a reagent in the kit can be a diluent for preparing the MLN4924 or a pharmaceutically acceptable salt thereof for administration. In some embodiments, a reagent in the kit can be a diluent for preparing the chemotherapeutic agent for administration. In some embodiments, a component in the kit can be a vessel for mixing the combination of MLN4924 and the chemotherapeutic agent. In some embodiments, the kit can include instructions for calculating the dose of each therapeutic component of the kit. In some embodiments, the instructions can include the Calvert formula.

[0095] In order that this present disclosure be more fully understood, the following examples are set forth. These examples are illustrative only and are not intended to limit the scope of the present disclosure in any way.

Examples

[0096] MLN4924, pharmaceutical compositions of MLN4924, processes for its synthesis, and polymorphic forms have been described previously. See, e.g., U.S. patent application Ser. No. 11/700,614 (Publ. No. 2007/0191293), Ser. No. 12/221,399 (Publ. No. 2009/0036678) and Ser. No. 12/779,331 (Publ. No. 2011/0021544) each of which is hereby incorporated by reference herein in its entirety. The hydrochloride salt of MLN4924 (((1 S,2 S,4R)-4-(4-((1S)-2,3-dihydro-1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate. HCl) was used for the experiments described below. The amounts listed reflect the amount of ((1S,2S,4R)-4-(4-((1S)-2,3-dihydro-1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]-pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate used.

1. In Vitro Cell Viability Assays

[0097] General Method: Cells are grown in their respective growth media, supplemented with 10% fetal bovine serum unless otherwise noted. A549— Ham's F-12K (Kaighn's) Medium; Calu-1 and HCT-116-McCoy's 5A Medium with 1% glutamine; NCI-H69, NCI-H82, NCI-H209, NCI-H510, NCI-H526, HARA, LK-2, LUDLU-1, NCI-H2170, NCI-H520, NCI-H1299, NCI-H1703, NCI-H596, RERF-LC-Sq1, and CHAGO-K-1—RPMI 1640 Medium; EBC-1, SK-MES-1, and VMRC-LCP—Minimum Essential Medium, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate; LC-1 Sq—1:1 mixture of RPMI-1640 and Ham's F12K (Kaighn's) Medium; SW900 and SW 1573—Leibovitz's L-15 (no CO_2); EPLC-272H-RPMI 1640 medium, 20% heat-inactivated FBS; KNS-62—Minimum Essential Medium, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 20% FBS; MIA PaCa-2—DMEM Medium, 10% FBS, 2.5% Horse serum, penicillin-streptomycin (pen/strep); BxPC-3—RPMI 1640 medium, 10% FBS, pen/strep; PANC1—DMEM medium, 10% FBS, pen/strep. The following number of cells are seeded per well of 384-well poly-D-lysine (PDL)-coated black, clear-bottom plates (BD BioCoat™) and allowed to adhere for 24 h at 37° C., 6% CO2: A549 and HCT116 (1000 cells/well); NCI-H69 (4000-7000); NCI-H82 (1500-2000); NCI-H209 and NCI-H510 (5000-7000); NCI-H526 (1500-2500); HARA, LK-2, CHAGO-K-1, NCI-H520, RERF-LC-Sq1, EBC-1, SK-MES-1, KNS-62, and VMRC-LCP (1000); Calu-1, EPLC-272H, and NCI-H1703 (1500); LUDLU-1, NCI-H596, SW900, SW 1573 and NCI-H2170 (2000); LC-1 Sq (4000); NCI-H1299 and MIA PaCa-2 (500); BxPC3 (750); PANC1 (1000 cells). ((1S,2S,4R)-4-(4-((1S)-2,3-Dihydro-1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate hydrochloride is dissolved in DMSO, and the chemotherapeutic agents are dissolved in either DMSO or PBS. The cells are treated with chemotherapeutic agents, either alone or in combination with MLN4924, at various doses for 48 h (HCT116), 72 h (A549, MIA PaCa-2, BxPC3, PANC1, HARA, LK-2, NCI-H520, RERF-LC-Sq1, EBC-1, SK-MES-1, VMRC-LCP, LUDLU-1, NCI-H2170, LC-1 Sq, CHAGO-K-1, Calu-1, EPLC-272H, KNS-62, NCI-H1299, NCI-H1703, NCI-H596, SW 1573, and SW900), or 96 hr (NCI-H69, NCI-H82, NCI-H209, NCI-H510, NCI-H526). A portion of each plate is used for positive controls (no agent is added), while another portion of each plate is used for negative controls (no cells are added). Viability is assessed with CellTiter-Glo® Cell Viability reagent used according to manufacturer's instructions (Promega). Luminescence is measured using a LEADseeker imaging system (GE Healthcare).

[0098] Statistical Analyses

[0099] Normalization.

[0100] The viability data is normalized separately for each plate by scaling the data so that the median of the negative controls is 0 and the median of the positive controls is 100. More formally,

$$V_i = 100\frac{U_i - \text{median}\ (U_-)}{\text{median}\ (U_+) - \text{median}\ (U_-)}$$

where V_i is the normalized viability of the i^{th} well, U_i is the raw viability measurement, median(U_-) is the median of the negative controls, and median(U_+) is the median of the positive controls. After normalization, the controls are discarded.

[0101] Response Surface Model and Fitting.

[0102] A response surface model similar to that of Minto et al. (C. F. Minto, et al., *Anesthesiology*, 2000, 92, 1603-1616)

is used to describe the relationship between the normalized viability and MLN4924 and chemotherapeutic agent concentrations. For a given plate, let

$$C=(C_A/I_1)+(C_B/I_2)$$

$$x=(C_A/I_1)/C$$

$$E_{max}=E_1+E_2x+E_3x^2+E_4x^3$$

$$I=1+I_3x(1-x)$$

$$S=S_1+S_2x+S_3x^2+S_4x^3$$

$$V=100-E_{max}(1+(I/C)^S)^{-1}+\text{error}$$

[0103] where E_1, E_2, E_3, E_4, I_1, I_2, I_3, S_1, S_2, S_3, and S_4 are parameters, C_A and C_B are the respective concentrations of agents A and B, and V is the normalized viability measurement. It is assumed that the error values are independent and identically distributed normal random variables. This model is an extension of the Hill equation (A. V. Hill, *J Physiol*., 1910, 40, iv-vii), which is commonly used to model the effect of a single agent. The data are fitted to this model using the maximum likelihood method with the statistical software program R (R Development Core Team, 2008, ISBN 3-900051-07-0, see, e.g., the R-Project for Statistical Computing website maintained by The R Foundation for Statistical Computing, hosted by Vienna University of Economics and Business, Vienna, Austria).

[0104] Quality Checks.

[0105] Three types of quality checks are applied to the plates. First, it is checked that the variation of the positive controls and the mean of the negative controls are small. Next, it is checked that new data agreed with data from previous single agent experiments. Finally, the residuals from the response surface fit are analyzed to ensure that the residual sum of squares is sufficiently small. All of these quality checks are based on numerical thresholds to make pass/fail decisions, and the same thresholds are used for all of the plates in the experiment. If a plate failed any one of the quality checks, it is removed from the analysis.

[0106] Measuring Synergy and Additivity.

[0107] The Combination Index (T. C. Chou and P. Talalay, *Adv. Enzyme Regul.*, 1984, 22:27-55) and Nonlinear Blending (J. J. Peterson and S. J. Novik, *Journal of Receptors and Signal Transduction,* 2007, 27:125-146.) are used as measures of agent synergy. The Combination Index is computed based on an isobologram, which is a slice of the dose response surface with constant viability. For the present analysis, the 50% isobologram, which is the dose contour that has 50% viability, is used. The $EC50_A$ and $EC50_B$ are defined be the respective doses of agents A and B alone that have a viability of 50%. For a point (D_A, D_B) along the 50% isobologram, the Combination Index is defined as $(D_A/EC50_A)+(D_B/EC50_B)$. Since the choice of ($D_A$, D_B) can be arbitrary, the constraint $D_A/D_B=EC50_A\ EC50_B$ is used.

[0108] In some cases, the Combination Index cannot be computed because the $EC50_A$ or $EC50_B$ does not exist. In such cases, Nonlinear Blending can be used as an alternative measure of synergy or additivity. Nonlinear Blending is found by considering a slice of the dose response surface that intersects both concentration axes and runs parallel to the viability axis. Let V_A and V_B be the viability where the slice intersects the drug A and B axes, respectively. Let V_{max} and V_{min} be the maximum and minimum viabilities along the slice. Let

$$NLB_S=\min(V_A,V_B)-V_{min}$$

$$NLB_A=V_{max}-\max(V_A,V_B)$$

Define the Nonlinear Blending value to be NLB_S if $NLB_S>NLB_A$ and $-NLB_A$ otherwise. Since the choice of the slice is arbitrary, the slice between the EC50 values (or the highest dose values, if the EC50s did not exist) of each drug alone is chosen. The standard error for both the Combination Index and the Nonlinear Blending are found using the Cramer-Rao lower bound (H Cramer, 1946. Mathematical Methods of Statistics; C. R. Rao, *Bulletin of the Calcutta Mathematical Society*, 1945, 37: 81-89).

[0109] Summarizing Replicates.

[0110] After completing the analysis of individual plates, the results are combined across the replicates. For a given measure and a set of replicates, the overall mean and standard error are computed using weighted averaging. A null mean, which corresponded to an additive effect, is then compared with the overall mean. The null mean is 1 for the Combination Index and 0 for Nonlinear Blending. Next, a two sized Z-test is performed based on the estimated mean and standard error. This produces a p-value for each measure and each cell line.

[0111] After computing the mean, standard error, and p-value for each set of replicates, these values require interpretation. Thus, a standard procedure is used to produce a classification (synergy, additivity, subadditivity, antagonism, or inconclusive) in each case. If the Combination Index exists for more than half of the replicates, then these measures are used to make the classification. If the Combination Index does not exist for a majority of the replicates, then a similar procedure based on Nonlinear Blending is used to make the classification.

[0112] Consider the case where the Combination Index is used to make the classification. If the p-value is greater than 0.05, the estimate for the Combination Index is not statistically different from 1. However, if the standard error is also very large, then the estimate is too uncertain to be informative. Hence, the classification is "Inconclusive". Otherwise, the classification is "Additivity". When the p-value is less than 0.05, the estimate for the Combination Index is statistically different from 1. However, if the mean is still close to 1, then the difference is not of practical significance. Thus, the result is classified based on the mean. A mean in the range (0.8, 1.2) is considered close enough to 1 to be classified as Additivity. A mean in the range (0, 0.8) is considered low enough to be classified as Synergy. A mean in the range (1.2, 2) is classified as Subadditivity because it indicates that combining the agents reduces the viability, but the reduction is less than what is predicted by the additive model. The threshold value of 2 can be derived from the definition of the Combination Index. A mean greater than 2 indicates that combining the agents increases the viability, so the combination is classified as Antagonism. Table 1 summarizes the decision rules for classifying the result based on the Combination Index. For the cases where Nonlinear Blending is used to classify the result, Table 2 describes a similar set of decision rules.

TABLE 1

Interpreting Combination Index. The Combination Index result is classified based on the p-value, the standard error, and the mean.

P-value	Standard error	Mean	Classification
>0.05	>0.25	Any	Inconclusive
>0.05	<0.25	Any	Additivity
<0.05	Any	0.8 to 1.2	Additivity
<0.05	Any	0 to 0.8	Synergy
<0.05	Any	1.2 to 2	Subadditivity
<0.05	Any	>2	Antagonism

TABLE 2

Interpreting Nonlinear Blending. The Nonlinear Blending result is classified in a manner similar to the Combination Index.

P-value	Standard error	Mean	Call
>0.05	>15	Any	Inconclusive
>0.05	<15	Any	Additivity
<0.05	Any	−15 to 15	Additivity

TABLE 2-continued

Interpreting Nonlinear Blending. The Nonlinear Blending result is classified in a manner similar to the Combination Index.

P-value	Standard error	Mean	Call
<0.05	Any	>15	Synergy
<0.05	Any	<−15	Antagonism

[0113] Results:

[0114] Cell viability assays performed according to the general method described above were used to assess the combination effect in vitro of MLN4924 (((1S,2S,4R)-4-(4-((1S)-2,3-dihydro-1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate hydrochloride; MLN4924 HCl was used in all experiments) with docetaxel, paclitaxel, gemcitabine, carboplatin, cisplatin, or oxaliplatin as outlined in Table 3. Gemcitabine combinations were evaluated in 6 cell lines (HCT116 colon; A549 NSCLC; and 3 pancreatic cell lines (PANC1, MIA PaCa-2, and BxPC3). Docetaxel, paclitaxel, cisplatin, carboplatin, and oxaliplatin were evaluated in HCT116, A549, and a selection of small cell lung cancer lines (SCLC) including NCI-H209, NCI-H510, NCI-H526, NCI-H82 and NCI-H69. Carboplatin was also evaluated in 19 additional NSCLC lines, some of which are classified as squamous NSCLC. The results were statistically analyzed as described above and the analysis are summarized in Table 3, which lists the Combination Index, Nonlinear Blending Score, and classification for the interaction which was assigned as described above and in Tables 1 and 2.

TABLE 3

Summary of the in vitro combination analysis

Table 3a: Taxane-based combinations

Agent	Cell line	Vehicle	Treatment time (hr)	Replicate experiments	Combination Index	Nonlinear Blending	Classification
docetaxel	A549	DMSO	72	1	1.00 ± 0.1	1 ± 1	Additivity
docetaxel	HCT116	DMSO	48	1	1.01 ± 0.09	7 ± 2	Additivity
docetaxel	NCI-H209	DMSO	96	1	1.27 ± 0.09	−16 ± 5	Subadditivity
docetaxel	NCI-H510	DMSO	96	1	1.15 ± 0.08	−13 ± 4	Additivity
docetaxel	NCI-H526	DMSO	96	1	1.38 ± 0.06	−23 ± 3	Subadditivity
docetaxel	NCI-H82	DMSO	96	1	1.45 ± 0.09	−11 ± 2	Subadditivity
paclitaxel	A549	DMSO	72	2	1.31 ± 0.07	−6 ± 0.9	Subadditivity
paclitaxel	HCT116	DMSO	48	4	1.44 ± 0.09	−14 ± 2	Subadditivity
paclitaxel	NCI-H209	DMSO	96	1	1.11 ± 0.07	−8 ± 3	Additivity
paclitaxel	NCI-H510	DMSO	96	1	1.29 ± 0.08	−22 ± 4	Subadditivity
paclitaxel	NCI-H526	DMSO	96	1	1.51 ± 0.08	−26 ± 3	Subadditivity
paclitaxel	NCI-H69	DMSO	96	1	2.20 ± 0.5	−16 ± 4	Antagonism
paclitaxel	NCI-H82	DMSO	96	1	1.67 ± 0.1	−16 ± 2	Subadditivity

Table 3a: Combination of MLN4924 with docetaxel or paclitaxel demonstrated additivity or subadditivity in 6 of 7 cell lines tested, with the exception of NCI-H69, in which the combination of MLN4924 and paclitaxel was antagonistic.

Table 3b: gemcitabine combinations

Agent	Cell line	Vehicle	Treatment time (hr)	Replicate experiments	Combination Index	Nonlinear Blending	Classification
gemcitabine	A549	DMSO	72	2	1.44 ± 0.07	−16 ± 3	Subadditivity
gemcitabine	HCT116	DMSO	48	3	0.49 ± 0.1	30 ± 3	Synergy
gemcitabine	PANC1	DMSO	72	1	0.24	19.4	Synergy
gemcitabine	BxPC3	DMSO	72	1	1.49	−26.0	Subadditivity
gemcitabine	MIA PaCa-2	DMSO	72	1	1.15	−8.1	Additivity

Table 3b. Combination of MLN4924 with gemcitabine demonstrated synergy in the HCT116 and PANC1 cell lines, additivity in the MIA PaCa-2 and subadditivity in the A549 and BxPC3 cell lines.

TABLE 3-continued

Summary of the in vitro combination analysis

Table 3c: platin-based combinations

Agent	Cell line	Vehicle	Treatment time (hr)	Replicate experiments	Combination Index	Nonlinear Blending	Classification
carboplatin	A549	PBS	72	3	0.72 ± 0.05	8 ± 4	Synergy
carboplatin	HCT116	PBS	48	2	0.71 ± 0.03	19 ± 2	Synergy
carboplatin	NCI-H209	DMSO	96	1	1.00 ± 0.06	−1 ± 1	Additivity
carboplatin	NCI-H510	DMSO	96	1	0.81 ± 0.08	5 ± 2	Additivity
carboplatin	NCI-H526	DMSO	96	1	1.02 ± 0.1	0 ± 2	Additivity
carboplatin	NCI-H69	DMSO	96	2	1.18 ± 0.09	−5 ± 1	Additivity
carboplatin	NCI-H82	DMSO	96	1	0.65 ± 0.1	12 ± 2	Synergy
cisplatin	A549	DMSO	72	3	0.65 ± 0.03	11 ± 5	Synergy
cisplatin	A549	PBS	72	2	0.75 ± 0.05	10 ± 1	Synergy
cisplatin	HCT116	DMSO	48	2	0.76 ± 0.02	21 ± 1	Synergy
cisplatin	HCT116	PBS	48	2	0.76 ± 0.09	14 ± 6	Synergy
cisplatin	NCI-H209	DMSO	96	1	1.28 ± 0.08	−15 ± 3	Subadditivity
cisplatin	NCI-H510	DMSO	96	1	NA	20 ± 3	Synergy
cisplatin	NCI-H69	DMSO	96	1	1.28 ± 0.2	−7 ± 3	Additivity
cisplatin	NCI-H82	DMSO	96	1	0.81 ± 0.07	10 ± 2	Additivity
oxaliplatin	A549	PBS	72	3	0.96 ± 0.06	2 ± 2	Additivity
oxaliplatin	HCT116	PBS	48	3	0.81 ± 0.09	10 ± 5	Additivity
oxaliplatin	NCI-H209	DMSO	96	1	1.12 ± 0.08	−5 ± 2	Additivity
oxaliplatin	NCI-H510	DMSO	96	1	0.94 ± 0.09	2 ± 3	Additivity
oxaliplatin	NCI-H526	DMSO	96	1	1.13 ± 0.09	−8 ± 4	Additivity
oxaliplatin	NCI-H69	DMSO	96	1	0.69 ± 0.1	11 ± 3	Synergy
oxaliplatin	NCI-H82	DMSO	96	1	0.85 ± 0.07	6 ± 2	Additivity
carboplatin	CALU-1	PBS	72	7	0.78 ± 0.05	11 ± 6	Synergy
carboplatin	CHAGO-K-1	PBS	72	2	0.88 ± 0.03	11 ± 1	Additivity
carboplatin	EBC-1	PBS	72	2	1.24 ± 0.06	−7 ± 1	Subadditivity
carboplatin	EPLC-272H	PBS	72	3	1.62 ± 0.03	−37 ± 2	Subadditivity
carboplatin	HARA	PBS	72	5	1.06 ± 0.1	−3 ± 10	Additivity
carboplatin	KNS-62	PBS	72	4	0.82 ± 0.1	10 ± 5	Additivity
carboplatin	LC-1-SQ	PBS	72	2	0.51 ± 0.04	28 ± 2	Synergy
carboplatin	LK-2	PBS	72	2	0.66 ± 0.05	15 ± 2	Synergy
carboplatin	LUDLU-1	PBS	72	3	0.84 ± 0.2	5 ± 5	Additivity
carboplatin	NCI-H1299	PBS	72	2	1.10 ± 0.05	−8 ± 2	Additivity
carboplatin	NCI-H1703	PBS	72	2	1.31 ± 0.02	−23 ± 2	Subadditivity
carboplatin	NCI-H2170	PBS	72	3	1.38 ± 0.1	−19 ± 7	Subadditivity
carboplatin	NCI-H520	PBS	72	2	NA	9 ± 20	Inconclusive
carboplatin	NCI-H596	PBS	72	3	0.59 ± 0.1	10 ± 7	Synergy
carboplatin	RERF-LC-SQ1	PBS	72	3	0.77 ± 0.06	11 ± 1	Synergy
carboplatin	SK-MES-1	PBS	72	2	1.32 ± 0.06	−22 ± 4	Subadditivity
carboplatin	SW1573	PBS	72	2	NA	6 ± 5	Additivity
carboplatin	SW900	PBS	72	2	0.85 ± 0.06	10 ± 1	Additivity
carboplatin	VMRC-LCP	PBS	72	3	1.00 ± 0.04	−3 ± 1	Additivity

Table 3c. Platin-based combinations with MLN4924: Combination of MLN4924 with carboplatin demonstrates synergy in 6 NSCLC cell lines (4 of which are squamous NSCLC), 1 colon, and 1 SCLC cell line, additivity in 4 other SCLC lines, additivity in 8 other NSCLC cell lines (7 of which are squamous NSCLC) and sub-additivity in 5 other NSCLC cell lines (3 of which are squamous NSCLC). Combination results for MLN4924 with carboplatin in 1 additional sqNSCLC cell line were inconclusive. Squamous NSCLC samples display 3q amplification in up to 94% of identified cases (Belvedere et al., Genomics, 2012; 99: 18-24). The following NSCLC cell lines did not demonstrate 3q amplification and thus may not represent squamous cell lines: A549, Calu-1, NCI-H2170, SK-MES-1, and SW900. Combination of MLN4924 with cisplatin demonstrates synergy in a NSCLC, colon, and SCLC line, with additivity or subadditivity in 3 other SCLC lines evaluated. Combination of MLN4924 with oxaliplatin demonstrates synergy in one SCLC line and additivity in a NSCLC, colon and 4 other SCLC lines. Each of the platin-based agents therefore demonstrated synergy with MLN4924 in at least one cell line.

2. In Vivo Tumor Efficacy Models

[0115] Tumor Models:

[0116] The PHTX02B breast xenograft model was established from a patient-derived tumor collected during surgery from a 51 year female with invasive ductal carcinoma classified as triple negative breast cancer (ER-/PR-/Her2-) by IHC. PHTX02B tumor fragments (approximately 2×2×3 mm^3) are implanted into the subcutaneous space in the right dorsal flank of female SCID-NOD mice (age 5-7 weeks, Jackson Laboratory, Bar Harbor, Me.) using a 13-gauge trocar.

[0117] NCI-H1650 (2×10^6) tumor cells in RPMI-1640 media are mixed with an equal volume of Matrigel (BD Biosciences) and aseptically injected into the subcutaneous space in the right dorsal flank of Balb/c Nude mice (age 4-6 weeks, Shanghai SINO-British SIPPR/BK Lab Animal Ltd.) using a 25-gauge needle.

[0118] NCI-H69 or NCI-H82 small cell lung tumor fragments (30- to 40-mg) are implanted into the subcutaneous space in the area of the right flank of NCr nu/nu mice (7-10 weeks, Charles River Laboratories, Inc, Frederick Md. or Wilmington, Mass.) using a 12-gauge trocar.

[0119] The PHTX249 Pa pancreatic xenograft model was established from a patient-derived tumor collected during surgery from a 63 year female with pancreatic adenocarcinoma. PHTX249 Pa tumor fragments (approximately 2×2×3 mm^3) are implanted into the subcutaneous space in the right dorsal flank of female CB-17 SCID mice (7-8 weeks, Taconic Farms, Inc., Cambridge City, Ind.) using a 13-gauge trochar.

[0120] LU1143 squamous non small cell lung cancer tumor fragments (approximately 2×2×3 mm^3) are implanted into the subcutaneous space in the flank of female BALB/C Nude mice (5 weeks, Shanghai Laboratory Animal Center, Shanghai, China).

[0121] LXFE409 squamous non small cell lung cancer tumor fragments are implanted into the subcutaneous space in the flank of female NMRI nu/nu mice (5-7 weeks, Janvier Labs, Saint Berthevin, France).

[0122] Test Agents:

[0123] All chemotherapeutic agents were clinical grade purchased from commercial sources and are administered as outlined below.

[0124] Docetaxel (Henry Schein, Inc., Pittsburgh Pa., Qilu Pharmaceutical Co., Ltd., Jinan, Shandong, China, and Sanofi-Aventis) is formulated in 0.9% saline and administered by intravenous injection (IV) once weekly (QW) at 5 mg/kg or 10 mg/kg.

[0125] Gemcitabine (Gemzar, Henry Schein, Inc, Pittsburgh Pa., and Myoderm Medical, Norristown, Pa. is formulated in 0.9% saline and administered by intraperitoneal injection (IP) q3D×4 (every 3 days for a total of 4 doses) at 2.5 mg/kg or 10 mg/kg, or by IV injection twice weekly (BIW) at 20 mg/kg IV

[0126] Cisplatin (PCH PHARMACHEMIE) is formulated in 0.9% saline and administered by IP injection q4D×3 (every 4 days for a total of 3 doses) at 2 mg/kg or 4 mg/kg.

[0127] Carboplatin (CARBOplatin Injection, Hospira, Inc., Qilu Pharmaceutical Co., Ltd. Jinan, Shandong, China, and Teva Gry-Pharma GmbH, Ulm, Germany) is formulated in 0.9% saline and administered by IP injection once weekly (QW) at 50 mg/kg. The once weekly schedule is also described as every 7 days (Q7D).

[0128] MLN4924 (MLN4924 hydrochloride salt; (((1S,2S,4R)-4-(4-((1S)-2,3-dihydro-1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate hydrochloride) is formulated in 10% HPbCD or 20% HPbCD in sterile water and administered by subcutaneous injection on one of the following schedules:

[0129] A. twice daily every day (BID);

[0130] B. twice daily for 5 days followed by 5 treatment-free days (BID 5 on/5 off); for example twice daily on days 1, 2, 3, 4, 5, 11, 12, 13, 14, 15

[0131] C. twice daily every 3 days for a total of 2 dosing days per week (BID Q3D×2/week); also described as BID BIW, or twice daily on a biweekly schedule; for example twice daily on days 1, 4, 8, 11, 15, 18

[0132] D. twice daily every 2 days for a total of 3 dosing days per week (BID Q2D×3/week); for example twice daily on days 1, 3, 5, 8, 10, 12, 15, 17, 19

[0133] E. once daily every 2 days for a total of 3 dosing days per week (Q2D×3/week); also described as three times a week (TIW); for example once daily on days 1, 3, 5, 8, 10, 12, 15, 17, 19.

[0134] Tumor Measurements:

[0135] Tumors are measured twice weekly using a vernier caliper. Tumor volumes are calculated using standard procedures ($0.5\times(\text{length}\times\text{width}^2)$). When the tumors reach a volume of approximately 130 mm^3 (LXFE409), 150 mm^3 (NCI-H1650, NCI-H69, NCI-H82),170 mm^3 (LU1143), 200 mm^3 (PHTX02B), or 225 mm^3 (PHTX249 Pa), mice are randomized into groups of 6-10 as described in the tables below, and injected with vehicle, MLN4924 (((1S,2S,4R)-4-(4-((1S)-2,3-dihydro-1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate hydrochloride salt; MLN4924 HCl was used in all experiments) or one of the agents (gemcitabine, docetaxel, cisplatin, or carboplatin), or the combination of MLN4924 and one of the agents, at various doses and schedules as described below in Tables 4-9. Tumor size and body weight are measured approximately twice a week for the duration of the study. Mice are euthanized when their tumor volume reached 10% of their body weight, or when the average tumor volume of a treatment or control group reached approximately 2000 mm^3. Tumor growth continued to be monitored after the dosing period in some studies. Tumor volume on study day 18, 21 or 22 for all groups of all studies is shown in Tables 4-9. Average tumor volume is reported as a function of time for selected groups of selected studies in Figures A, B, and C.

[0136] Statistical Analyses of Combination Effect for Tumor Growth in Subcutaneous Xenograft Models.

[0137] Measurements from day 0 to 18, 21 or 22 are analyzed as specified in Tables 4-9, All tumor volumes have a value of 1 added to them before $\log_{10}$ transformation. For each animal, the log tumor volume at day 0 is subtracted from the log tumor volume on the subsequent days. This difference vs. time is used to calculate an area under the curve (AUC) for each animal using the trapezoid rule. In instances when an animal in a treatment group is removed early from the study, the last observed tumor value is carried forward through all subsequent time points. The synergy score for the combination of agents A and B is defined as

$$100*(\text{mean}(AUC_{AB})-\text{mean}(AUC_A)-\text{mean}(AUC_B)+\text{mean}(AUC_{ctl}))/\text{mean}(AUC_{ctl}):$$

where AUC_{AB}, AUC_A, AUC_B, and AUC_{ctl} are the AUC values for animals in the combination group, the A group, the B group, and the control group, respectively. The standard error of the synergy score is computed based on the variation in the AUC values among the animals. A two sided t-test is used to determine if the synergy score is significantly different from zero. If the P-value is above 0.05, then the combination is considered to be additive. If the P-value is below 0.05, and the synergy score is less than zero, then the combination is considered to be synergistic. If the P-value is below 0.05, the synergy score is greater than zero, and the combination is more effective than either agent alone, then the combination is considered to be subadditive. Otherwise, the combination is classified as antagonistic.

[0138] Results:

[0139] Mouse xenograft models, performed as described in the general methods above, were used to assess the combination effect in vivo of MLN4924 and docetaxel, MLN4924 and gemcitabine, MLN4924 and carboplatin, and MLN4924 and cisplatin. The details for each study are as shown below in Tables 4-9. The results were analyzed using the statistical analysis described above and the classification of the combination is shown below in Tables 4-9. MLN4924 hydrochloride salt; (((1S,2S,4R)-4-(4-((1S)-2,3-dihydro-1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate hydrochloride) was used in all experiments; the values listed in table 4-9 reflect the amounts of MLN4924.

[0140] MLN4924 and Docetaxel

[0141] In the PHTX02B breast xenograft model (shown in FIG. **1**), dosing of the single agents (MLN4924 SC 60 mg/kg BID 5 days on/5 days off) and docetaxel (5 mg/kg IV QW) inhibited tumor growth compared to the control vehicle group. However, tumors in the single agent groups continued to grow in size during the treatment period. The combination treatment using these doses and schedules led to complete inhibition of tumor growth with a decrease in tumor volume compared to the starting volume. All treatment groups from the study are shown in Table 4a. The combination benefit for this combination in this study was scored as additive (Table 4b).

TABLE 4a

Combination of docetaxel and MLN4924 in PHTX02B xenograft model

Group	Treatment	Dosing Regimen	Route	average tumor volume day 21	SEM tumor volume day 21	number of mice in group (number on day 21)
1	10% HPbCD	BID	SC	2066.3	87.8	10
2	30 mg/kg MLN4924	BID	SC	894.4	83	10
3	5 mg/kg docetaxel	QW	IV	736.3	112.2	10
4	30 mg/kg MLN4924; 5 mg/kg docetaxel	BID; QW	SC; IV	359.2	45.9	10 (9)
5	30 mg/kg MLN4924	BID 5 on/5 off	SC	1110.9	150.2	10
6	30 mg/kg MLN4924; 5 mg/kg docetaxel	BID 5 on/5 off; QW	SC; IV	444.8	73.5	10
7	60 mg/kg MLN4924	BID 5 on/5 off	SC	532.2	88.4	10
8	60 mg/kg MLN4924; 5 mg/kg docetaxel	BID 5 on/5 off; QW	SC; IV	97.5	26.5	10

TABLE 4b

Classification for in vivo combination of docetaxel and MLN4924 in PHTX02B xenograft model

Treatment	Synergy score (Day 21)	Synergy score standard error	P-value	Classification
MLN4924 30 mg/kg BID + docetaxel	10	9.8	0.317	additive
MLN4924 30 mg/kg BID 5 on/5 off + docetaxel	11.8	12.6	0.356	additive
MLN4924 60 mg/kg BID 5 on/5 off + docetaxel	−21.8	15.2	0.165	additive

[0142] The combination of docetaxel and MLN4924 was also evaluated in the NCI-H1650 NSCLC model, the LU1143 sqNSCLC model, and the LXFE409 sqNSCLC model and results are shown in Tables 5a-5e. In the LXFE409 sqNSCLC model, the combination of MLN4924 (120 mg/kg SC Q2D× 3/week for 3 weeks) with docetaxel (10 mg/kg IV QW for 3 weeks) resulted in tumor regression in all 8 mice treated with this regimen, and complete regressions were maintained in this combination treatment group through the end of study on day 95. In contrast, tumors treated with the single agents MLN4924 or docetaxel regrew after the treatment period ended. A graph of these results is shown in FIG. **4**.

TABLE 5a

Combination of docetaxel and MLN4924 in NCI-H1650 xenograft model

Group	Treatment	Dosing Regimen	Route	average tumor volume day 21	SEM tumor volume day 21	number of mice in group (number on day 21)
1	20% HPbCD; 0.9% Saline	Q2Dx3/week; QW	SC; IV	990.8	68.1	8
2	120 mg/kg MLN4924	Q2Dx3/week	SC	774.2	38.3	8
3	90 mg/kg MLN4924	Q2Dx3/week	SC	776.8	44.8	8
4	10 mg/kg docetaxel	QW	IV	282.7	44.4	8
5	5 mg/kg docetaxel	QW	IV	719.2	49.1	8
6	120 mg/kg MLN4924; 10 mg/kg docetaxel	Q2Dx3/week; QW	SC; IV	154.5	21.8	8
7	120 mg/kg MLN4924; 5 mg/kg docetaxel	Q2Dx3/week; QW	SC; IV	429.1	35.5	8
8	90 mg/kg MLN4924; 10 mg/kg docetaxel	Q2Dx3/week; QW	SC; IV	212.8	44.3	8
9	90 mg/kg MLN4924; 5 mg/kg docetaxel	Q2Dx3/week; QW	SC; IV	586.4	38.1	8

TABLE 5b

Classification for in vivo combination of docetaxel and MLN4924 in NCI-H1650 xenograft model

Treatment	Synergy score (Day 21)	Synergy score standard error	P-value	Classification
120 mg/kg MLN4924; 5 mg/kg docetaxel	−16.2	11.4	0.175	additive
120 mg/kg MLN4924; 5 mg/kg docetaxel	−11.5	7.6	0.145	additive
90 mg/kg MLN4924; 10 mg/kg docetaxel	−7.2	12.7	0.578	additive
90 mg/kg MLN4924; 5 mg/kg docetaxel	1.4	7.5	0.852	additive

TABLE 5f

Classification for in vivo combination of docetaxel and MLN4924 in LXFE409 xenograft model

Treatment	Synergy score (Day 21)	Synergy score standard error	P-value	Classification
120 mg/kg MLN4924; 10 mg/kg docetaxel	49.2	25	0.069	additive

[0145] MLN4924 and Gemcitabine

[0146] In the PHTX02B xenograft model (shown in FIG. **2**), dosing of the single agents (MLN4924 SC 30 mg/kg BID) and gemcitabine (10 mg/kg IP q3d×4) inhibited tumor growth compared to the control vehicle group, but tumors in the single agent groups continued to grow in size during the treatment period. In contrast, the combination treatment prevented tumor growth, and tumor volume at the end of the treatment period remained the same as the starting volume. All treatment groups from the study are shown in Table 6a. The combination benefit was assessed as additive (Table 6b).

TABLE 5c

Combination of docetaxel and MLN4924 in LU1143 xenograft model

Group	Treatment	Dosing Regimen	Route	average tumor volume day 21	SEM tumor volume day 21	number of mice in group (number on day 21)
1	20% HPbCD; 0.9% saline	BID; QW	SC; IV	996.1	128	8
2	45 mg/kg MLN4924	BID	SC	801.2	112.4	8
3	10 mg/kg docetaxel	QW	IV	498.9	58.5	8
5	45 mg/kg MLN4924; 10 mg/kg docetaxel	BID; QW	SC; IV	366.5	37.3	8

[0143] Groups 4 and 6 from this study in the LU1143 xenograft model contained carboplatin and are presented in Table 7c. Group 1 (vehicle control) and group 2 (MLN4924) are also presented in Table 7c.

TABLE 5d

Classification for in vivo combination of docetaxel and MLN4924 in LU1143 xenograft model

Treatment	Synergy score (Day 21)	Synergy score standard error	P-value	Classification
45 mg/kg MLN4924; 10 mg/kg docetaxel	2.8	11.4	0.808	additive

[0147] In the PHTX249 Pa xenograft model, dosing of gemcitabine (20 mg/kg IV BIW) inhibited tumor growth compared to the control vehicle group, but tumors treated with MLN4924 (90 mg/kg SC BID BIW) did not show growth inhibition compared to the control vehicle group. The combination treatment inhibited tumor growth to a similar extent as gemcitabine alone, and the combination effect was assessed as additive. Treatment groups and combination analysis are shown in Tables 6c and 6d.

TABLE 5e

Combination of docetaxel and MLN4924 in LXFE409 xenograft model

Group	Treatment	Dosing Regimen	Route	average tumor volume day 21	SEM tumor volume day 21	number of mice in group (number on day 21)
1	20% HPbCD	Q2Dx3/week	SC	1561.6	167.2	8
3	120 mg/kg MLN4924	Q2Dx3/week	SC	167.1	23.3	8
6	10 mg/kg docetaxel	QW	IV	44.6	14.5	8
7	120 mg/kg MLN4924; 10 mg/kg docetaxel	Q2Dx3/week; QW	SC; IV	10.3	4.6	8

[0144] Groups 2, 4, and 5 from this study in the LXFE409 xenograft model are relevant to the combination of MLN4924 with carboplatin and are presented in Table 7e. Group 1 (vehicle control) is also presented in Table 7e.

TABLE 6a

Combination of gemcitabine and MLN4924 in PHTX02B xenograft model

Group	Treatment	Dosing Regimen	Route	average tumor volume day 22	SEM tumor volume day 22	number of mice in group (number on day 22)
1	10% HPbCD	BID	SC	1661.9	66.9	10
2	2.5 mg/kg gemcitabine	Q3Dx4	IP	1386.3	113.9	10
3	10 mg/kg gemcitabine	Q3Dx4	IP	769.4	30.2	10 (9)
4	30 mg/kg MLN4924	BID	SC	647.4	78.6	10
5	30 mg/kg MLN4924; 2.5 mg/kg gemcitabine	BID; Q3Dx4	SC; IP	499.5	66.5	10
6	30 mg/kg MLN4924; 10 mg/kg gemcitabine	BID; Q3Dx4	SC; IP	196.2	34.5	10 (8)

TABLE 6b

Classification for in vivo combination of gemcitabine and MLN4924 in PHTX02B xenograft model

Treatment	Synergy score (Day 22)	Synergy score standard error	P-value	Classification
30 mg/kg MLN4924; 2.5 mg/kg gemcitabine	0.7	13.4	0.956	additive
30 mg/kg MLN4924; 10 mg/kg gemcitabine	−21.8	16.1	0.188	additive

TABLE 6c

Combination of gemcitabine and MLN4924 in PHTX249Pa xenograft model

Group	Treatment	Dosing Regimen	Route	average tumor volume day 18	SEM tumor volume day 18	number of mice in group (number on day 18)
1	0.9% saline	QW	IV	1430.4	183.5	6
2	20 mg/kg gemcitabine	BIW	IV	585.1	85	6
3	90 mg/kg MLN4924	BID BIW	SC	1748.9	432.2	6 (4)
4	90 mg/kg MLN4924; 20 mg/kg gemcitabine	BID BIW; BIW	IV; SC	621.8	153.5	6

TABLE 6d

Classification for in vivo combination of gemcitabine and MLN4924 in PHTX249Pa xenograft model

Treatment	Synergy score (Day 18)	Synergy score standard error	P-value	Classification
90 mg/kg MLN4924; 20 mg/kg gemcitabine	−11.9	22.1	0.6	additive

[0148] MLN4924 and Platins

[0149] In the NCI-H69 xenograft model (shown in FIG. **3**), dosing of the single agents (MLN4924 SC 120 mg/kg BID Q3D×2/week) and carboplatin (50 mg/kg IP QW) inhibited tumor growth compared to the control vehicle group. However, tumors in the single agent groups continued to grow in size during the treatment period. In contrast, the combination treatment using these doses and schedules led to complete inhibition of tumor growth with a decrease in tumor volume compared to the starting volume. All treatment groups from the study are shown in Table 7a. The combination benefit for this treatment was scored as synergy (Table 7b). The combination of carboplatin and MLN4924 was further evaluated in 2 sqNSCLC xenograft models, LU1143 (Table 7c,d) and LXFE409 (Table 7e,f). The combination of cisplatin and MLN4924 was evaluated in the NCI-H69 xenograft model (Tables 8a,b) and in the NCI-H82 xenograft model (Tables 9a,b).

TABLE 7a

Combination of carboplatin and MLN4924 in NCI-H69 xenograft model

Group	Treatment	Dosing Regimen	Route	average tumor volume day 21	SEM tumor volume day 21	number of mice in group (number on day 21)
1	20% HPbCD; 0.9% saline	BID Q3D x 2/week; QW	SC; IP	949.8	157.6	8
2	120 mg/kg MLN4924	BID Q3D x 2/week	SC	415.2	103.8	8
3	60 mg/kg MLN4924	BID Q3D x 2/week	SC	597.1	55.9	8
4	50 mg/kg carboplatin	QW	IP	501.3	87.4	8
5	120 mg/kg MLN4924; 50 mg/kg carboplatin	BID Q3D x 2/week; QW	SC; IP	115.2	23.5	8
6	60 mg/kg MLN4924; 50 mg/kg carboplatin	BID Q3D x 2/week; QW	SC; IP	247.5	25.4	8

TABLE 7b

Classification for combination of carboplatin and MLN4924 in NCI-H69 xenograft model

Treatment	Synergy score (Day 21)	Synergy score standard error	P-value	Classification
120 mg/kg MLN4924; 50 mg/kg carboplatin	−63.1	19.8	0.005	synergy

TABLE 7b-continued

Classification for combination of carboplatin and MLN4924 in NCI-H69 xenograft model

Treatment	Synergy score (Day 21)	Synergy score standard error	P-value	Classification
60 mg/kg MLN4924; 50 mg/kg carboplatin	−43.8	16.5	0.016	synergy

TABLE 7c

Combination of carboplatin and MLN4924 in LU1143 xenograft model

Group	Treatment	Dosing Regimen	Route	average tumor volume day 21	SEM tumor volume day 21	number of mice in group (number on day 21)
1	20% HPbCD; 0.9% saline	BID; QW	SC; IV	996.1	128	8
2	45 mg/kg MLN4924	BID	SC	801.2	112.4	8
4	50 mg/kg carboplatin	QW	IP	658.7	66.8	8
6	45 mg/kg MLN4924; 50 mg/kg carboplatin	BID; QW	SC; IP	275.8	53.2	8

TABLE 7d

Classification for combination of carboplatin and MLN4924 in LU1143 xenograft model

Treatment	Synergy score (Day 21)	Synergy score standard error	P-value	Classification
45 mg/kg MLN4924; 50 mg/kg carboplatin	−26.3	16.1	0.118	additive

TABLE 7e

Combination of carboplatin and MLN4924 in LXFE409 xenograft model

Group	Treatment	Dosing Regimen	Route	average tumor volume day 21	SEM tumor volume day 21	number of mice in group (number on day 21)
1	20% HPbCD	Q2Dx3/week	SC	1561.6	167.2	8
2	90 mg/kg MLN4924	Q2Dx3/week	SC	707.5	194.2	8
4	50 mg/kg carboplatin	QW	IP	204.4	96.8	8
5	90 mg/kg MLN4924; 50 mg/kg carboplatin	Q2Dx3/week; QW	SC; IP	122.5	54.6	8

TABLE 7f

Classification for combination of carboplatin and MLN4924 in LXFE409 xenograft model

Treatment	Synergy score (Day 21)	Synergy score standard error	P-value	Classification
90 mg/kg MLN4924; 50 mg/kg carboplatin	37.8	20.4	0.081	additive

TABLE 8a

Combination of cisplatin and MLN4924 in NCI-H69 xenograft model

Group	Treatment	Dosing Regimen	Route	average tumor volume day 22	SEM tumor volume day 22	number of mice in group (number on day 22)
1	20% HPbCD; 0.9% saline	BID Q2Dx3/week; Q4Dx3	SC; IP	983.2	184.6	10 (9)
2	60 mg/kg MLN4924	BID Q2Dx3/week	SC	475.4	90.6	10
3	45 mg/kg MLN4924	BID Q3Dx2/week	SC	438.5	92.8	10
4	4 mg/kg Cisplatin	Q4Dx3	IP	213.6	19.3	10
5	60 mg/kg MLN4924; 4 mg/kg cisplatin	BID Q2Dx3/week; Q4Dx3	SC; IP	55.3	10.8	10
6	45 mg/kg MLN4924; 4 mg/kg cisplatin	BID Q3Dx2/week; Q4Dx3	SC; IP	98.7	27.4	10 (8)

TABLE 8b

Classification for combination of cisplatin and MLN4924 in NCI-H69 xenograft model

Treatment	Synergy Score (Day 22)	Synergy score standard error	P-value	Classification
60 mg/kg MLN4924; 4 mg/kg cisplatin	−27.5	15.6	0.091	additive

TABLE 8b-continued

Classification for combination of cisplatin and MLN4924 in NCI-H69 xenograft model

Treatment	Synergy Score (Day 22)	Synergy score standard error	P-value	Classification
45 mg/kg MLN4924; 4 mg/kg cisplatin	−1.1	28.2	0.97	additive

TABLE 9a

Combination of cisplatin and MLN4924 in NCI-H82 xenograft model

Group	Treatment	Dosing Regimen	Route	average tumor volume day 22	SEM tumor volume day 22	number of mice in group (number on day 22)
1	20% HPbCD; 0.9% saline	BID Q2Dx3/week; Q4Dx3	SC; IP	946.9	135.2	10 (9)
2	60 mg/kg MLN4924	BID Q2Dx3/week	SC	861.9	121	10
3	60 mg/kg MLN4924	BID Q3Dx2/week	SC	783.3	97.8	10
4	4 mg/kg cisplatin	Q4Dx3	IP	445.2	57	10
5	2 mg/kg cisplatin	Q4Dx3	IP	628.7	72	10 (9)
6	60 mg/kg MLN4924; 4 mg/kg cisplatin	BID Q2Dx3/week; Q4Dx3	SC, IP	194.4	65.3	10 (8)
7	60 mg/kg MLN4924; 4 mg/kg cisplatin	BID Q3Dx2/week; Q4Dx3	SC; IP	199.1	39.6	10 (9)
8	60 mg/kg MLN4924; 2 mg/kg cisplatin	BID Q2Dx3/week; Q4Dx3	SC; IP	438.3	49.3	10 (8)
9	60 mg/kg MLN4924; 2 mg/kg cisplatin	BID Q3Dx2/week; Q4Dx3	SC; IP	503.2	51.1	10

TABLE 9b

Classification for combination of cisplatin and MLN4924 in NCI-H82 xenograft model

Treatment	Synergy score (Day 22)	Synergy score standard error	P-value	Classification
60 mg/kg MLN4924 BID Q2Dx3/week; 4 mg/kg cisplatin	−57	14.1	0.001	synergy
60 mg/kg MLN4924 BID Q3Dx2/week; 4 mg/kg cisplatin	−25.4	13.4	0.072	additive
60 mg/kg MLN4924 BID Q2Dx3/week; 2 mg/kg cisplatin	−12	11.8	0.319	additive
60 mg/kg MLN4924 BID Q3Dx2/week; 2 mg/kg cisplatin	9.7	9.4	0.312	additive

1. A method of treating a solid tumor, comprising administering to a patient in need of such treatment a combination of ((1S,2S,4R)-4-(4-((1S)-2,3-dihydro-1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-hydroxycyclopentyl) methyl sulfamate or a pharmaceutically acceptable salt thereof, and one or more of:

i) a platin

ii) a taxane; or

iii) gemcitabine.

2. The method of claim **1**, wherein the solid tumor is breast cancer, colon cancer, lung cancer, pancreatic cancer, esophageal cancer, bladder cancer, cholangiocarcinoma, or head and neck cancer.

3. The method of claim **1**, wherein the platin is cisplatin, carboplatin, oxaliplatin, satraplatin, picoplatin, nedaplatin or triplatin.

4. The method of claim **1**, wherein the taxane is paclitaxel, docetaxel or nab-paclitaxel.

5. The method of claim **1**, wherein the ((1S,2S,4R)-4-(4-((1S)-2,3-dihydro-1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate or a pharmaceutically acceptable salt thereof is administered on each of days 1, 3, and 5 of a 21 day schedule.

6. The method of claim **1**, wherein the ((1S,2S,4R)-4-(4-((1S)-2,3-dihydro-1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate or a pharmaceutically acceptable salt thereof is administered on each of days 1, 8, and 15 of a 28 day schedule.

7. The method of claim **1**, comprising administering to a patient in need of such treatment a combination of ((1S,2S,4R)-4-(4-((1S)-2,3-dihydro-1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]-pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate or a pharmaceutically acceptable salt thereof, and a taxane.

8. The method of claim **7**, wherein the taxane is docetaxel.

9.-**10**. (canceled)

11. The method of claim **8**, wherein the docetaxel is administered on day 1 of a 21 day schedule and the amount of docetaxel administered is 75 mg/m^2.

12. (canceled)

13. The method of claim **1**, comprising administering to a patient in need of such treatment a combination of ((1S,2S,4R)-4-(4-((1S)-2,3-dihydro-1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]-pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate or a pharmaceutically acceptable salt thereof, and a platin.

14. The method of claim **13**, wherein the platin is cisplatin or carboplatin.

15. (canceled)

16. The method of claim **5**, wherein the amount of ((1S,2S,4R)-4-(4-((1S)-2,3-dihydro-1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate or a pharmaceutically acceptable salt thereof administered on each of days 1, 3, and 5 of a 21 day schedule is less than or equal to 50 mg/m^2.

17. The method claim **1**, comprising administering to a patient in need of such treatment a combination of ((1S,2S,4R)-4-(4-((1S)-2,3-dihydro-1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate or a pharmaceutically acceptable salt thereof, a platin, and a taxane.

18. The method of claim **17**, wherein the taxane is paclitaxel.

19. The method of claim **33**, wherein the carboplatin and paclitaxel are administered on day 1 of a 21 day schedule.

20. The method of claim **1**, comprising administering to a patient in need of such treatment a combination of ((1S,2S,4R)-4-(4-((1S)-2,3-dihydro-1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]-pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate or a pharmaceutically acceptable salt thereof, and gemcitabine.

21. (canceled)

22. The method of claim **6**, wherein the amount of ((1S,2S,4R)-4-(4-((1S)-2,3-dihydro-1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate or a pharmaceutically acceptable salt thereof administered on each of days 1, 8, and 15 of a 28 day schedule is less than or equal to 100 mg/m^2.

23. The method claim **20**, wherein the amount of gemcitabine administered on each of days 1, 8, and 15 of a 28 day schedule is 1000 mg/m^2.

24.-**27**. (canceled)

28. A kit for treating a solid tumor in a subject in recognized need thereof comprising:

at least one medicament comprising at least one dose of ((1S,2S,4R)-4-(4-((1S)-2,3-dihydro-1H-inden-1-ylamino)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate or a pharmaceutically acceptable salt thereof, and

at least one medicament comprising at least one dose of one or more of:

i) a platin

ii) a taxane; or

iii) gemcitabine

or a pharmaceutically acceptable salt thereof;

said kit for treating solid tumors further comprising dosing instructions for administering the medicaments for treatment of the subject in recognized need thereof.

29.-**32**. (canceled)

33. The method of claim **18**, wherein the platin is carboplatin.

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