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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
2 July 2009 (02.07.2009)

PCT

(10) International Publication Number
WO 2009/082686 A1

(51) International Patent Classification:
A01N 57/00 (2006.01)

(21) International Application Number:
PCT/US2008/087623

(22) International Filing Date:
19 December 2008 (19.12.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/016,032 21 December 2007 (21.12.2007) US

(71) Applicant *(for all designated States except US)*: **PROLX PHARMACEUTICALS CORP.** [US/US]; 221 E 6th Street, Tucson, Arizona 85705 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **KIRKPATRICK, Lynn** [US/US]; 326 E Cowan Drive, Houston, Texas 77007 (US).

(74) Agent: **MILLER, Raymond A.**; Pepper Hamilton LLP, One Mellon Center, 500 Grant Street, 50th Floor, Pittsburgh, Pennsylvania 15219 (US).

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

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

Published:
— *with international search report*

(54) **Title:** COMPOSITIONS AND METHODS FOR USING ASYMMETRIC DISULFIDES

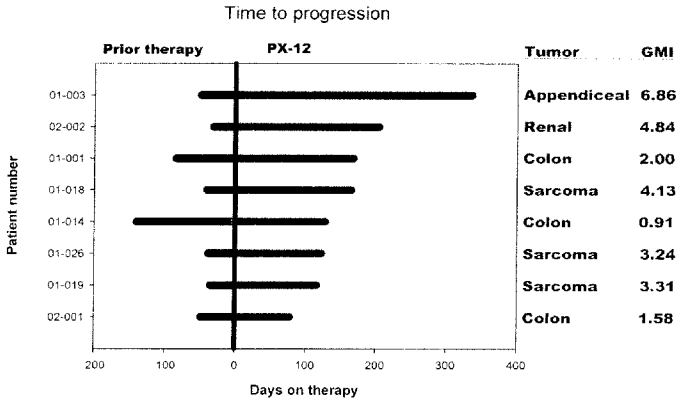


FIGURE 1

(57) **Abstract:** Embodiments of the present invention provide compositions and methods of treating cancer comprising administering a thioredoxin inhibitor, such as an asymmetric disulfide, over an extended period of time.

WO 2009/082686 A1

A. Title: Compositions and Methods for Using Asymmetric Disulfides

B. Cross-Reference to Related Applications:

This application claims priority to U.S. Provisional Patent Application Ser. No. 61/016,032, filed December 21, 2007; the disclosure of which is incorporated by reference herein in its entirety.

C. Government Interests: Not applicable

D. Parties to a Joint Research Agreement: Not applicable

E. Incorporation by Reference of Material submitted on a Compact Disc: Not applicable

F. Background

1. **Field of Invention:** Not applicable
2. **Description of Related Art:** Not applicable

G. Brief summary of the invention

[0001] Embodiments of the invention described herein include methods for treating cancer, including the step of administering a therapeutically effective amount of a thioredoxin inhibitor over an extended period of time. In certain embodiments the thioredoxin inhibitor may be administered over about 36 hours to about 96 hours. In certain embodiments, the thioredoxin inhibitor may be administered over about a 72 hour period. In some embodiments, the administration may be carried out intravenously, and in particular embodiments, the administration may be carried out by intravenous infusion. In various embodiments, the administration may occur about every fourteen to about every twenty-eight days, and in some embodiments, the administration occurs about every twenty-one days.

[0002] In some embodiments, a therapeutically effective amount of the thioredoxin inhibitor is from about 100 $mg/m^2/day$ to about 1000 $mg/m^2/day$. In other embodiments, a therapeutically effective amount of the thioredoxin inhibitor is from about 200 $mg/m^2/day$ to about 700 $mg/m^2/day$; and in still other embodiments, the therapeutically effective amount is from about 300 $mg/m^2/day$ to about 600 $mg/m^2/day$. In certain embodiments, the therapeutically effective amount is about 300 $mg/m^2/day$, about 400 $mg/m^2/day$, about 500 $mg/m^2/day$ or about 600 $mg/m^2/day$.

[0003] The methods of some embodiments may be used to treat metastatic cancer, and in particular embodiments, the cancer may be breast cancer, cervical cancer, colorectal cancer, gastric cancer, hepatoma, hepatocellular cancer, gastrointestinal cancer, liver cancer, lung cancer,

myeloma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, small-cell lung cancer, and squamous cell cancer.

[0004] In certain embodiments, the method may further include the step of administering a therapeutically effective amount of a secondary agent, and in at least one embodiment, the secondary agent is an antithrombotic, such as coumadin. In other embodiments, the secondary agent is a chemotherapeutic agent, for example, cisplatin, tamoxifen or paclitaxel.

[0005] In particular embodiments, the thioredoxin inhibitor is an asymmetric disulfide, and in certain embodiments, the thioredoxin inhibitor is 1-methylpropyl 2-imidazolyl disulfide (PX-12), or a pharmaceutically acceptable salt thereof.

[0006] Further embodiments of the present invention provide sustained release or controlled release pharmaceutical compositions comprising a thioredoxin inhibitor, preferably, an asymmetric disulfide, including 1-methylpropyl 2-imidazolyl disulfide (PX-12).

H. Description of Drawings

[0007] For a fuller understanding of the nature and advantages of the present invention, reference should be had to the following detailed description taken in connection with the accompanying drawings, in which:

[0008] **Fig.** 1 shows a time to progression of patients receiving PX-12 versus the most recent prior therapy. Growth Modulation Index (GMI) is the ratio of time to progression on PX-12 versus time to progression on prior therapy.

[0009] **Fig. 2** shows a one-hour PX-12 infusion results in a decrease in plasma Trx within 15 minutes of infusion; degree and duration of decrease was found to increase with dose. PD: Trx levels pre-dosing; IF: Trx levels 15 minutes into infusion.

[0010] **Fig. 3** shows a 3 hour PX-12 infusion (right panel) is more effective than 1 hour PX-12 infusion (left panel) in inducing an immediate decrease in plasma TRX levels. EI: Trx levels at end of infusion; PD: Trx levels before dosing.

I. Detailed Description

[0011] Before the present compositions and methods are described, it is to be understood that this invention is not limited to the particular processes, compositions, or methodologies described, as these may vary. It is also to be understood that the terminology used in the description is for the purpose of describing the particular versions or embodiments only, and is

not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0012] *Optical Isomers--Diastereomers--Geometric Isomers—Tautomers.* Compounds described herein may contain an asymmetric center and may thus exist as enantiomers. Where the compounds according to the invention possess two or more asymmetric centers, they may additionally exist as diastereomers. The present invention includes all such possible stereoisomers as substantially pure resolved enantiomers, racemic mixtures thereof, as well as mixtures of diastereomers. The formulas are shown without a definitive stereochemistry at certain positions. The present invention includes all stereoisomers of such formulas and pharmaceutically acceptable salts thereof. Diastereoisomeric pairs of enantiomers may be separated by, for example, fractional crystallization from a suitable solvent, and the pair of enantiomers thus obtained may be separated into individual stereoisomers by conventional means, for example, by the use of an optically active acid or base as a resolving agent or on a chiral HPLC column. Further, any enantiomer or diastereomer of a compound of the general formula may be obtained by stereospecific synthesis using optically pure starting materials or reagents of known configuration.

[0013] It must also be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to a "cell" is a reference to one or more cells and equivalents thereof known to those skilled in the art, and so forth.

[0014] As used herein, the term "alkyl" is meant to refer to a saturated hydrocarbon group which is straight-chained or branched. Example alkyl groups include, but are not limited to, methyl (Me), ethyl (Et), propyl (*e.g.*, n-propyl and isopropyl), butyl (*e.g.*, n-butyl, isobutyl, t-butyl), pentyl (e.g., n-pentyl, isopentyl, neopentyl), and the like. An alkyl group can contain

from 1 to about 20, from 2 to about 20, from 1 to about 10, from 1 to about 8, from 1 to about 6, from 1 to about 4, or from 1 to about 3 carbon atoms.

[0015] As used herein, "aryl" refers to monocyclic or polycyclic (e.g., having 2, 3 or 4 fused rings) aromatic hydrocarbons such as, for example, phenyl, naphthyl, anthracenyl, phenanthrenyl, indanyl, indenyl, and the like. In some embodiments, aryl groups have from 6 to about 20 carbon atoms. In some embodiments, aryl groups have from 6 to about 10 carbon atoms.

[0016] As used herein, "arylalkyl" refers to a C_{1-6} alkyl substituted by aryl.

[0017] As used herein, "halo" or "halogen" includes fluoro, chloro, bromo, and iodo.

[0018] As used herein, "alkoxy" refers to an -O-alkyl group. Example alkoxy groups include methoxy, ethoxy, propoxy (*e.g.*, n-propoxy and isopropoxy), t-butoxy, and the like.

[0019] As used herein, "amino" refers to NH_2.

[0020] As used herein, "nitro" refers to NO_2.

[0021] As used herein, "hydroxyalkyl" refers to an alkyl group substituted by a hydroxyl group. An example is $-CH_2OH$ or $-CH_2CH_2OH$.

[0022] As used herein, the term "optionally substituted" means that substitution is optional and therefore includes both unsubstituted and substituted atoms and moieties. A "substituted" atom or moiety indicates that any hydrogen on the designated atom or moiety can be replaced with a selection from the indicated substituent group, provided that the normal valency of the designated atom or moiety is not exceeded, and that the substitution results in a stable compound. For example, if a methyl group (i.e., CH_3) is optionally substituted, then 3 hydrogen atoms on the carbon atom can be replaced with substituent groups.

[0023] As used herein, the term "continuous infusion" means the administration of a therapeutic into a blood vessel, usually over a prolonged period of time.

[0024] As used herein, the term "about" means plus or minus 10% of the numerical value of the number with which it is being used. Therefore, about 50% means in the range of 45%-55%.

[0025] As used herein, the term "day" means about 24 hours.

[0026] "Administering" when used in conjunction with a therapeutic means to administer a therapeutic directly into or onto a target tissue or to administer a therapeutic to a patient, whereby the therapeutic positively impacts the tissue to which it is targeted.

[0027] The term "animal" as used herein includes, but is not limited to, humans and non-human vertebrates such as wild, domestic and farm animals.

[0028] The term "improves" is used to convey that the present invention changes either the appearance, form, characteristics and/or the physical attributes of the tissue to which it is being provided, applied or administered. For example, the change in form may be demonstrated by any of the following alone or in combination: decrease in cell proliferation associated with cancer, reduction in tumor size, inhibition or reduction of metastasis, prolonged survival, etc.

[0029] As used herein, the term "inhibiting" means preventing the onset of one or more symptoms of a a disease, disorder, or condition., alleviating or ameliorating one or more symptoms of a disease, disorder, or condition, or eliminating a disease, disorder, or condition.

[0030] As used herein, a "kit" refers to one or more pharmaceutical compositions and instructions for administration or prescription of the one or more compositions. The instructions may consist of product insert, instructions on a package of one or more pharmaceutical compositions, or any other instruction.

[0031] By "pharmaceutically acceptable", it is meant the carrier, diluent or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

[0032] The present invention also includes pharmaceutically acceptable salts of the compounds described herein. As used herein, "pharmaceutically acceptable salts" refers to derivatives of the disclosed compounds wherein the parent compound is modified by converting an existing acid or base moiety to its salt form. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts of the present invention include the conventional non-toxic salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. The pharmaceutically acceptable salts of the present invention can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile (ACN) are preferred. Lists of suitable salts are found in *Remington's*

Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418 and *Journal of Pharmaceutical Science*, 66, 2 (1977), each of which is incorporated herein by reference in its entirety.

[0033] As used herein, the term "sustained release" or "controlled release" formulation means a pharmaceutical formulation that provides a therapeutically effective amount of the active agent over an extended period of time, preferably at least about 24 hours, more preferably more than about 24 hours, more preferably about 36 hours or more, such as about 72 hours or 72 hours or more.

[0034] As used herein, the term "therapeutic" means an agent utilized to treat, combat, ameliorate, prevent or improve an unwanted condition or disease of a patient. In part, embodiments of the present invention are directed to the treatment of cancer or the decrease in proliferation of cells.

[0035] A "therapeutically effective amount" or "effective amount" of a composition is a predetermined amount calculated to achieve the desired effect, *i.e.*, to inhibit, block, or reverse the activation, migration, or proliferation of cells. The activity contemplated by the present methods includes both medical therapeutic and/or prophylactic treatment, as appropriate. The specific dose of a compound administered according to this invention to obtain therapeutic and/or prophylactic effects will, of course, be determined by the particular circumstances surrounding the case, including, for example, the compound administered, the route of administration, and the condition being treated. However, it will be understood that the effective amount administered will be determined by the physician in light of the relevant circumstances including the condition to be treated, the choice of compound to be administered, and the chosen route of administration, and therefore, the above dosage ranges are not intended to limit the scope of the invention in any way. A therapeutically effective amount of compound of this invention is typically an amount such that when it is administered in a physiologically tolerable excipient composition, it is sufficient to achieve an effective systemic concentration or local concentration in the tissue.

[0036] The terms "treat," "treated," or "treating" as used herein refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological condition, disorder or disease, or to obtain beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results

include, but are not limited to, alleviation of symptoms; diminishment of the extent of the condition, disorder or disease; stabilization (*i.e.*, not worsening) of the state of the condition, disorder or disease; delay in onset or slowing of the progression of the condition, disorder or disease; amelioration of the condition, disorder or disease state; and remission (whether partial or total), whether detectable or undetectable, or enhancement or improvement of the condition, disorder or disease. Treatment includes eliciting a clinically significant response without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment.

[0037] Generally speaking, the term "tissue" refers to any aggregation of similarly specialized cells which are united in the performance of a particular function.

[0038] Thioredoxin-1 (Trx-1) is a low molecular weight (10-12 kDa) redox protein present in both prokaryotic and eukaryotic cells. Trx-1 was originally studied for its ability to act as a reducing cofactor for ribonucleotide reductase, the first unique step in DNA synthesis. More recently, thioredoxin has been shown to provide redox control over a number of transcription factors and to modulate their binding to DNA, thus regulating gene transcription. Transcription factors regulated by Trx-1 include NF-κB, the glucocorticoid receptor, and AP1 (or Fos/Jun heterodimer) (indirectly through a redox factor Ref-1(HAPE). Trx-1 is a protooncogene that transforms mouse NIH 3T3 embryonic cells allowing them to form tumors in immunodeficient nude mice. Trx-1 transfection of cancer cells stimulates tumor growth and inhibits both spontaneous and drug induced apoptosis. The conserved Cys^{32}-Gly-Pro-Cys^{35}-Lys active site of human Trx-1 undergoes reversible oxidation–reduction of the cysteine residues catalyzed by the NAPDH-dependent flavoprotein thioredoxin reductase Trx-1. Redox activity is essential for the biological effects of Trx-1 as transfection with a redox inactive mutant Trx-1 fails to transform the cells. Moreover, it inhibits cell growth, potentiates apoptosis and blocks tumor formation by the cells in immunodeficient *scid* mice.

[0039] Trx-1 protein levels are significantly elevated in several human primary cancers including gastric cancer (50% of cases), colorectal cancer (55%), pancreatic cancer (41%), lung cancer (50%) and breast cancer (52%). Primary gastric cancer has shown highly significant correlations between increased Trx-1 expression, increased proliferation and resistance to cancer drug induced apoptosis. Studies in colorectal cancer and non-small cell lung cancer have shown that increased Trx-1 expression is an independent prognostic factor for decreased patient

survival. Taken together, these results suggest that Trx-1 has an important role in maintaining the transformed phenotype of some human cancers as well as their resistance to chemotherapeutic drugs and is, thus, a highly rational target for novel cancer drug development.

[0040] Trx-1 is found both in the cytoplasm and nucleus and is secreted into the plasma via a leaderless pathway. Exogenous thioredoxin has been found to act as a growth factor itself, and can also act as a co-cytokine. Trx-1 is secreted by tumors into plasma. The circulating levels of Trx-1 in patients with cancer have been reported to be over twice that found in healthy controls.

[0041] PX-12 (1-methylpropyl 2-imidazolyl disulfide) is a small molecule inhibitor of thioredoxin-1 that has been shown to be a potent *in vitro* inhibitor of Trx-1. PX-12 binds to intracellular Trx-1 in tumor cell lines and inhibits white blood cell Trx-1 when added to fresh whole blood samples and inhibits signaling by irreversibly binding to the Cys^{73} non-catalytic site of Trx-1. *In vivo*, PX-12 stimulates apoptosis and inhibits growth of human tumor xenografts in *scid* mice. Combination studies have shown that PX-12 enhances the anti-tumor activity of clinically-relevant cytotoxic agents such as cisplatin, tamoxifen and paclitaxel. In pharmaceutics studies, PX-12 was found to be extremely stable with a projected shelf-life of approximately 7 years, and ongoing real-time stability studies currently demonstrate 100% stability of PX-12 over two years at 4°C.

[0042] Drugs that block thioredoxin may provide a unique approach to cancer therapy. PX-12 inhibits the growth-promoting properties of thioredoxin and has shown excellent anti-tumor activity in animal models. For example, in a phase I trial, PX-12 delivered as a 3 hour infusion was well tolerated and demonstrated hints of anti-tumor activity and pharmacodynamic activity across a wide dosage range. A subsequent phase Ib study demonstrated that delivering PX-12 via a 24 hour infusion is also well tolerated.

[0043] Preclinical GLP studies in rats and dogs were also performed. Male and female CDF®(F-344)/CrlBR rats (10 animals/sex/group) received intravenous bolus injections of dose preparations containing vehicle control or PX-12 at 5, 15, or 30 mg/kg body weight/day (mg/kg/day) on Days 1 through 5. The dose volume was 15 mL/kg. Nine animals died during the first week of the study (on Day 1, six males in the 30 mg/kg/day cohort; on Day 2, one male in the control vehicle group; on Day 5, one male and one female in the 15 mg/kg/day cohort). In addition, two females in the 30 mg/kg/day cohort were sacrificed on Days 5 and 19, respectively,

because of excessive tail irritation. Clinical observations during the treatment phase for surviving animals in the 15 or 30 mg/kg/day cohorts included one or more of the following: hunched or pale appearance, ataxia, hypoactivity, irregular respiration, clear oral or nasal discharge, or lesions and abnormalities affecting the tails. At lethal doses, additional clinical observations included convulsions and recumbancy. Most observations, including weight loss, were not noted after Day 8 and none of these observations were noted after Day 29, indicating recovery from treatment with the test material.

[0044] Four groups of beagle dogs were administered PX-12 or carrier (aqueous PEG 400) via intravenous bolus injection for 5 days at doses of 0, 2.5, 7.5, and 15 mg/kg/day. All animals survived until study termination. No significant hematologic, renal or hepatic abnormalities were noted. Clinical signs including vocalization, excretion (urine and feces), labored respiration, rapid respiration, excessive salivation, ocular and/or nasal discharge, and red or reddened conjunctiva, gums, and/or ears were observed during dosing on multiple days and/or in beagles dosed at levels of 5, 12.5, and 17.5 mg/kg/day in a second study (100 to 350 $mg/m^2/day$). The severity and number of clinical signs were dose-related. Due to the severity of the clinical signs,,dosing was not completed on multiple days for the female dog receiving 12.5 mg/kg/day and the male dog receiving 17.5 mg/kg/day. Localized swelling was observed in the dogs receiving 17.5 mg/kg/day and was considered to be due to extravasation caused by the study drug's formulation.

[0045] The original phase I study of PX-12 in patients with advanced metastatic cancer has been completed and one phase II study in patients with advanced pancreatic cancer and one phase Ib study in patients with advanced gastric cancer remain ongoing.

[0046] *Phase I Trial.* Thirty-eight patients with advanced metastatic cancer were treated with PX-12 in a dose-escalating, open-label phase I trial. PX-12 was given intravenously via continuous infusion over 1 or 3 hours, daily for 5 days, and was then repeated every 21 days. Over the course of the trial, 142 cycles of PX-12 were administered with a median of 2 cycles and an average of 3.7 cycles per patient. For individual patients, the number of cycles ranged from 1 to 14. PX-12 administered daily for 5 days was tolerated with minimal toxicity at doses ranging from 9 to 226 mg/m^2. Intravenous infusion of solutions of 2.5 mg/mL PX-12 have been delivered safely via a central line with no noted inflammation at delivery site. Metabolism of PX-12 releases a low molecular weight thiol, 2-butanethiol, an irritant, which is given off in

expired breath. The study demonstrated that a cough and odor related to the expiration of the metabolite is dose related. The odor may be experienced for approximately 1-2 hours after administration and a cough may develop within the first 5-10 minutes of PX-12 infusion. Grade 1-2 cough, bronchial irritation, flushing, sweating, headache, anorexia, fatigue, nausea, vomiting, taste disturbance and Grade 3 hypoxia may be experienced with the administration of PX-12. Grade 3 hypoxia (during Cycle 1) and Grade 2 reversible bilateral pneumonitis (during Cycle 2) were dose limiting toxicities observed at the 300 mg/m^2 dose level in two separate patients. A cough and odor associated with drug metabolite expiration limited the tolerability of treatment via the 3 hour infusion. A Maximally Tolerated Dose (MTD) of 226 mg/m^2/day IV over 3 hours was determined in this first study. The more prolonged 3 hour infusion delivery was beneficial in reducing the Trx-1 levels over the 21 day cycle.

[0047] Among 38 patients with advanced cancer treated with PX-12 at different dose levels, there was one response (45% reduction in tumor mass) in a patient with appendiceal cancer. Six patients (including patients with colon cancer, renal cell carcinoma and sarcoma) exhibited stable disease which persisted from 6 to 11 months. Clinical benefit was also assessed by comparing the time to progression (TTP) on PX-12 treatment versus TTP on the patient's prior therapy. The ratio between these measurements has been defined as the Growth Modulation Index (GMI). **Fig. 1** illustrates the GMI for 8 patients that achieved prolonged disease stabilization on PX-12. As predicted from the preclinical studies, administration of PX-12 led to a dose-dependent decrease in plasma Trx-1 levels.

[0048] *Other Phase Ib.* A phase Ib study in patients with gastrointestinal cancers in which patients are treated with IV PX-12 via continuous infusion over 24 hours every other week was also completed. Following treatment of fourteen patients using this regimen, a revision was made to the protocol, and the study is currently enrolling patients to be treated with a 24 hour infusion every week.

[0049] The 24 hour infusion trial with PX-12 was initiated to evaluate the tolerability of PX-12, it's safety, it's pharmacokinetic and pharmacodynamic properties (Trx-1 inhibition as measured by decreased Trx-1 levels in plasma) and any efficacy (stable disease, partial or complete responses). The original schedule involved providing a patient with an infusion pump and PX-12 in solution to deliver 150 mg/m^2/24hrs to 450 mg/m^2/24hrs on a 14 day cycle. After 14 patients had been treated (the cohort of 450 mg/m^2/24hrs was complete) the protocol was

modified to allow delivery of PX-12 via continous infusion over 24 hours every 7 days due to the limited stability of disease and limited thioredoxin inhibition observed in the patients enrolled in the 14 day cycle cohort. These 14 patients and an additional 4 patients, have been enrolled and will receive PX-12 over a 24 hour period every 7 days.

[0050] The 24 hour infusion was determined to be well tolerated, as there have been few adverse events reported in the study to date. Additionally, the 450 mg/m^2/day dose of PX-12, which has been successfully delivered, is greater then the maximum deliverable daily dose of 300 mg/m^2/day determined during the Phase I trial in which PX-12 was delivered over a 3 hour infusion. However, little efficacy was noted in the 24 hour infusion trial, whether given every 14 or 7 days. Out of 18 patients receiving PX-12 as a 24 hour infusion via either schedule, there were only 2 patients with measurable SD as compared to the 7 durable SD and 1 minor response out of 38 patients in the Phase I trial that delivered PX-12 as a 1 or 3 hour infusion daily for 5 days on a 21 day schedule. Moreover, the 7 durable SD patients exhibited a decrease in circulating thioredoxin (Trx-1) levels as measured by ELISA. No dose-limiting toxicity has been observed and only modest clinical benefit was noted in two patients with defined SD. Persistent reductions in thioredoxin levels were observed for these patients on Day 28 prior to cycle 3 (44 and 59% drop from pretreatment levels). The study was modified to increase the frequency of the 24 hour administration to every 7 days.

[0051] The 24 hour infusion delivery appears to be more tolerable than the 3 hour infusion. However, to achieve greater efficacy, a higher exposure to PX-12 may be required. Hence, it was planned to deliver PX-12 over a 72 hour period at doses ranging from 100 mg/m^2/day to 1000 mg/m^2/24 hour, a delivery schedule that can be tolerated and is anticipated to achieve greater efficacy. The clinical benefit and target inhibition also appears suboptimal in the current phase Ib 24 hour infusion trial. To increase total drug exposure over a 21 day cycle, this 72 hour infusion study was planned with the anticipation of achieving better target inhibition and potentially better efficacy with this delivery schedule.

[0052] *Phase II.* The phase II study in patients with pancreatic cancer involves the continuous infusion of PX-12 over 3 hours on days 1-5 of a 21 day cycle. Patients were randomized to one of 2 dose levels: 54 mg/m^2 or 128 mg/m^2.

[0053] *Preclinical and Clinical Pharmacokinetics of PX-12.* Studies in mice indicate that PX-12 is either rapidly metabolized or rapidly distributed following intravenous injection.

Less than 1% of the total calculated intravenously injected dose of PX-12 was present in mouse plasma 5 minutes after injection. Stability studies in freshly isolated plasma (not stored) show that PX-12 is immediately reduced to inactive thiol metabolites at low concentrations. However, when increasing amounts of PX-12 are added to plasma, some parent drug can be detected although initial analytical methodology was limited during these studies.

[0054] Blood samples for pharmacokinetic and pharmacodynamic analyses were collected from patients enrolled in the phase I clinical trial. Samples were collected on Days 1 and 5 before treatment, 15 minutes into the first infusion, and up to 240 minutes after the first infusion. In addition, samples were collected before treatment and 1 minute prior to the end of infusion on Days 2-4 of the first cycle. Plasma samples were divided for analysis of Trx-1 levels for a pharmacodynamic endpoint assay and for pharmacokinetic analysis of PX-12 and its non-toxic and inactive metabolite 2-mercaptoimidazole (2-MI). Initial pharmacokinetic analyses indicated that no PX-12 was detected at any dose levels although methodology for detection was not optimized. New more analytical methodology has been developed which indicate that PX-12 can be detected. These samples are currently being reanalyzed. 2-MI was detected in patients treated with PX-12 at concentrations of 36 mg/m^2 and above which appears to be dose dependent. However, accumulation of 2-MI was not observed throughout a 5 day treatment cycle.

[0055] *Preclinical and Clinical Pharmacodynamic of PX-12.* PX-12 lowers the circulating level of thioredoxin in as little as 2 hours after intravenous injection in *scid* mice. In addition, administration of PX-12 is associated with a significant decrease in VEGF and a decrease in vascular permeability as monitored by dynamic contrast enhanced MRI.

[0056] Clinical pharmacodynamic studies were conducted in the phase I clinical trial. Plasma Trx-1 levels were measured by ELISA assay. Samples were collected before treatment and 2-4 hours after the end of the infusion, and on days 8, 15 and 21. These studies showed that plasma Trx-1 levels were rapidly lowered within the first 15 minutes of infusion initiation, even at the lowest dose level (9 mg/m^2), and remained low through the infusion. In those patients receiving a 1 hour infusion, plasma Trx-1 levels were observed to return to pretreatment levels in a dose-dependent manner with the rate of return being faster at lower doses as described in **Fig. 2**.

[0057] Trx-1 levels were reduced, and a decreased level of Trx-1 in the circulation was maintained for more extended periods of time when PX-12 was administered as a 3 hour infusion. Pretreatment levels of Trx-1 were compared to Trx-1 levels at the end of infusion and expressed as percent change. This analysis showed that the 3 hour infusion provided a more consistent decrease in circulating Trx-1 than did the 1 hour delivery. **Fig. 3** shows that 3 hour delivery of PX-12 at doses of 54 mg/m^2 to 96 mg/m^2 lowered plasma Trx-1 by 25% to 75%. Similar results were observed in the cohorts receiving 128 to 300 mg/m^2 with average dose-dependent decreases of 50% to 95% when comparing pretreatment Trx-1 levels with those measured 21 days after treatment.

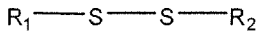
[0058] Plasma VEGF levels were measured before treatment and 120 or 240 minutes following infusion of PX-12. Compared to pretreatment levels, plasma levels of VEGF were reduced by approximately 50% in samples collected 240 minutes after infusion in 10 of the 33 patients tested.

[0059] Various embodiments of the invention described herein are directed to a method for administering a thioredoxin inhibitor such as, for example, an asymmetric disulfide. In some embodiments, the asymmetrical disulfides have respective R groups of divergent functionality. In some embodiments, the thioredoxin inhibitor is a compound of the general formula R_1-S--S-R_2, wherein one of R_1 or R_2 is a good leaving group and the other is a poor leaving group. Examples of good leaving groups are compounds which contain electron withdrawing groups or groups which delocalize the electrons of the functional groups (i.e., aromatic and imidazlyl groups). In some embodiments, the aromatic groups of the present invention include heteroatoms such as oxygen, nitrogen, and sulfur. Poor leaving groups do not generally have such electron withdrawing characteristics or delocalized electrons. Thus, they do not form substantially stable species when or if they are cleared from the molecule. An example of a poor leaving group is an unsubstituted alkane or alkyl group. Other asymmetrical disulfides may be (bis) asymmetrical disulfides. In some embodiments, the asymmetric disulfides of the present invention include imidazolyl disulfide, thiadiazolyl disulfide, mercaptothiadiazolyl disulfide, thiazolyl disulfide, phenyl disulfide, benzyl disulfide, phenylethyl disulfide, nicotinic acid disulfide, pyrimidine disulfide, benzoxazolyl disulfide, benzothiazolyl disulfide, benzimidazolyl disulfide, purinyl disulfide, cycloalkyl disulfide, captopril disulfide, and menthone disulfide or any pharmaceutically acceptable salts thereof. In some embodiments, the methods of the present

invention may generally include the step of administering the thioredoxin inhibitor over an extended period of time. In certain embodiments, an extended period of time is over about a 36 hour to about a 96 hour period. In further embodiments, an extended period of time is about a 72 hour period. In some embodiments, the asymmetric disulfide may be benzyl 2-imidazolyl disulfide or 1-methylpropyl 2-imidazolyl disulfide (PX-12). In some embodiments, the thioredoxin inhibitor may be administered in any form, including but not limited to intravenously, orally, etc. In some embodiments, the thioredoxin inhibitor is administered intravenously.

[0060] Methods encompassed by some embodiments may further include repeating a 72 hour administration any number of times. For example, in some embodiment, the administration may occur about every 21 days. In other embodiments, the administration cycle may include administration over from about 36 hours to about 96 hours once every 14 days or once every 28 days. In other embodiments, the administration cycle may be over about 72 hours once every 14 days or once every 28 days. The administration cycle may vary depending on the patient and it is well within the purview of the artisan skilled in the medical arts to determine a proper administration cycle to achieve the most beneficial effect.

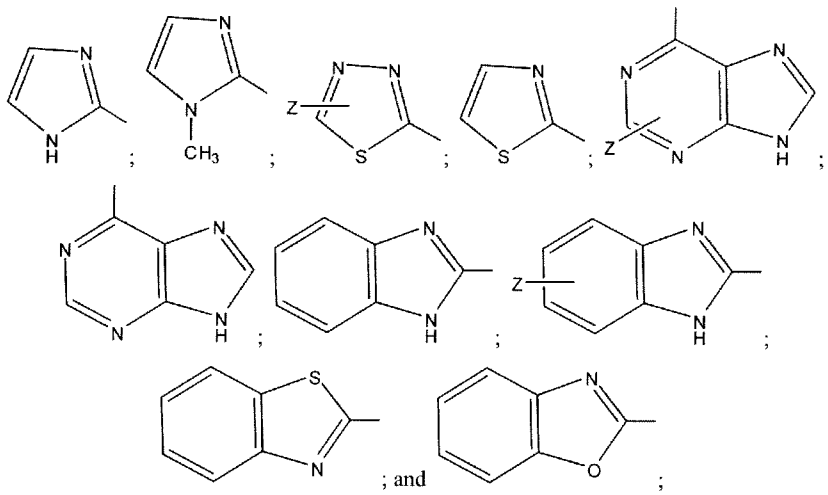
[0061] Some embodiments provide methods for treating cancer comprising administering a therapeutically effective amount of a compound of formula I:



I

wherein:

R_1 is a moiety selected from the group consisting of:



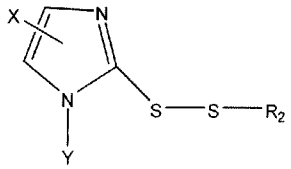
; and ;

R_2 is alkyl, aryl, or arylalkyl, wherein said alkyl, aryl, and arylalkyl groups are optionally substituted by C_1-C_6 alkyl, alkoxy, or -COOH; and

Z is C_1-C_6 alkyl, -SH, amino, or nitro;

via continuous infusion over from about 48 to about 72 hours.

[0062] Other embodiments of the present invention provide methods for treating cancer comprising administering a therapeutically effective amount of a compound of formula II:



II

wherein:

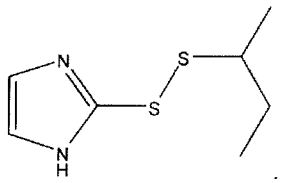
R_2 is C_1-C_6 alkyl or arylalkyl;

X is H, halo, alkyl, alkoxy, or amino; and

Y is H, alkyl, hydroxyalkyl, or arylalkyl;

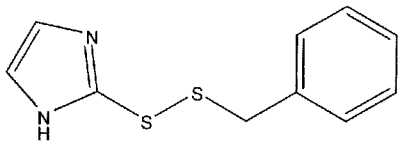
via continuous infusion over from about 48 to about 72 hours. In some embodiments, the continuous infusion is over about 72 hours.

[0063] In further embodiments, the compound of formula II is a compound of formula II(a):



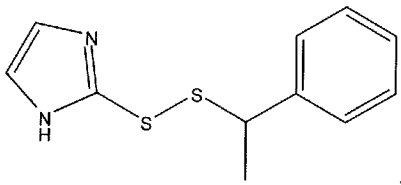
II(a)

[0064] In yet other embodiments, the compound of formula II is a compound of formula II(b):



II(b)

[0065] In some embodiments, the compound of formula II is a compound of formula II(c):



II(c)

[0066] In some embodiments, the continuous infusion is repeated about every fourteen to about every twenty-eight days. In other embodiments, the continuous infusion is repeated about every twenty-one days.

[0067] Other embodiments of the invention provide methods wherein the therapeutically effective amount of a compound of formula I is from about 300 mg/m^2 to about 3000 mg/m^2. In other embodiments, the therapeutically effective amount of a compound of formula II is from about 300 mg/m^2 to about 3000 mg/m^2. Still other embodiments provide methods wherein the therapeutically effective amount of a compound of formula II is from about 600 mg/m^2 to about

2100 mg/m^2. Yet other embodiments provide methods wherein the therapeutically effective amount of a compound of formula II is from about 900 mg/m^2 to about 1800 mg/m^2. Further embodiments provide methods wherein said therapeutically effective amount of a compound of formula II is about 900 mg/m^2. Some embodiments provide methods wherein the therapeutically effective amount of a compound of formula II is about 1200 mg/m^2. In some embodiments, the therapeutically effective amount of a compound of formula II is administered via continuous infusion at a rate of about 400 mg/m^2/24 hours. In other embodiments, the therapeutically effective amount of a compound of formula II is about 1500 mg/m^2.

[0068] Specific modes of administration may vary and may depend on the indication. The selection of the specific route of administration and the dosing regimen may be adjusted or titrated by the clinician according to methods known to the clinician in order to obtain the optimal clinical response. The amount of compound to be administered is that amount which is therapeutically effective. The dosage to be administered will depend on the characteristics of the subject being treated, *e.g.*, the particular animal treated, age, weight, health, types of concurrent treatment, if any, and frequency of treatments, and can be easily determined by one of skill in the art (*e.g.*, by the clinician). In various embodiments, an effective amount of a thioredoxin inhibitor delivered during each administration cycle may range from about 100 mg/m^2/day to about 1000 mg/m^2/day. In some embodiments, an effective amount may be about 200 mg/m^2/day to about 700 mg/m^2/day, and in other embodiments, an effective amount may be about 300 mg/m^2/day to about 600 mg/m^2/day. In particular embodiments, an effective amount may be about 300 mg/m^2/day, about 400 mg/m^2/day, about 500 mg/m^2/day, or about 600 mg/m^2/day. Based on the effective amounts described above, the total amount of thioredoxin inhibitor delivered over the course of a 72 hour administration may vary from about 300 mg/m^2 to about 3000 mg/m^2, from about 600 mg/m^2 to about 2100 mg/m^2, and from about 900 mg/m^2 to about 1800 mg/m^2. In yet other embodiments, an effective amount of the thioredoxin inhibitor may vary as treatment progresses. For example, a dosing regimen may be increased or decreased as treatment proceeds through administration cycles, or the daily dosage may increase or decrease during a 72 hour administration.

[0069] In some embodiments, the dosing regimen as described above may be combined with a secondary form of treatment or a secondary agent. For example, in one embodiment, the thioredoxin inhibitor may be administered concurrently or during ongoing treatment with another

antitumor or chemotherapeutic agent such as, but not limited to, cisplatin, tamoxifen and paclitaxel. In some embodiments, the thioredoxin inhibitor may enhance the efficacy of the secondary agent or provide a cumulative effect. In other embodiments, the thioredoxin inhibitor may be administered by an intravenous infusion via a central venous catheter. In some embodiments, an effective amount of an antithrombotic agent, such as, for example, coumadin may be administered concurrently with the placement of the port and continued while the central catheter is in place.

[0070] Some of the embodiments described herein may be used to treat any proliferative disorder known in the art or identified in the future, including but not limited to, cancer. Any form of cancer may be treated using such methods. However, in some embodiments the cancer may include breast cancer, cervical cancer, colorectal cancer, gastric cancer, hepatoma, hepatocellular cancer, gastrointestinal cancer, liver cancer, lung cancer, myeloma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, small-cell lung cancer, and squamous cell cancer. In some embodiments, the cancer may be metastatic. Of course, in the case of metastatic and/or certain aggressive forms of cancer, several of these indications may be treated simultaneously using the methods described herein.

[0071] Various other embodiments include pharmaceutical compositions. For example, in one embodiment, the invention may include a pharmaceutical composition comprising a thioredoxin inhibitor, as defined above, and a pharmaceutically acceptable carrier, excipient or diluent. In other embodiments, a pharmaceutical composition may include an effective amount of a thioredoxin inhibitor and a pharmaceutically acceptable carrier, excipient or diluent.

[0072] Some further embodiments of the present invention provide sustained release or controlled release pharmaceutical compositions comprising a thioredoxin inhibitor, preferably, an asymmetric disulfide, including 1-methylpropyl 2-imidazolyl disulfide (PX-12). In some embodiments, the sustained release pharmaceutical composition provides a therapeutically effective amount of the thioredoxin inhibitor, such as an asymmetric disulfide, including, for example, PX-12, for more than 24 hours, such as about 72 hours.

[0073] Additional embodiments of the present invention provide a kit comprising one or more pharmaceutical compositions comprising a thioredoxin inhibitor, preferably an asymmetric disulfide, such as PX-12, and instructions for administering or prescribing the one or more pharmaceutical compositions, comprising directions to administer or prescribe the one or

more pharmaceutical compositions in an amount sufficient to result in administration of a therapeutically effective amount of the composition over an extended period of time to a patient. Yet further embodiments of the present invention provide kits comprising one or more pharmaceutical compositions according to any of the previous embodiments of the compositions described herein, or any combination thereof, and instructions for administering or prescribing the one or more pharmaceutical compositions, comprising directions to administer or prescribe the one or more pharmaceutical compositions according to the embodiments of the methods described herein, or any combination thereof. In certain embodiments, the pharmaceutical composition may be a sustained release composition comprising a thioredoxin inhibitor, e.g., an asymmetric disulfide such as PX-12, and instructions for administering or prescribing the one or more pharmaceutical compositions, comprising directions to administer or prescribe the one or more pharmaceutical compositions in an amount sufficient to result in administration of a therapeutically effective amount of the composition over an extended period of time to a patient.

[0074] The compounds of the present invention can be administered in the conventional manner by any route where they are active. Administration can be systemic or local. For example, administration can be, but is not limited to, parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, oral, buccal, or ocular routes, or intravaginally, by inhalation, by depot injections, or by implants. In some embodiments, the administration may be parenteral or intravenous. Thus, modes of administration for the compounds of the present invention (either alone or in combination with other pharmaceuticals) can be injectable (including short-acting, depot, implant and pellet forms injected subcutaneously or intramuscularly).

[0075] In some embodiments, pharmaceutical formulations containing the compounds of the present invention and a suitable carrier can be in various forms including, but not limited to, tablets, solutions, powders, fluid emulsions, fluid suspensions, semi-solids, and dry powder comprising an effective amount of a thioredoxin inhibitor of the invention. It is also known in the art that the active ingredients can be contained in such formulations with pharmaceutically acceptable diluents, fillers, disintegrants, binders, lubricants, surfactants, hydrophobic vehicles, water soluble vehicles, emulsifiers, buffers, humectants, moisturizers, solubilizers, preservatives and the like. The means and methods for administration are known in the art and an artisan can refer to various pharmacologic references for guidance. For example, *Modern Pharmaceutics*,

Banker & Rhodes, Marcel Dekker, Inc. (1979); and *Goodman & Gilman's, The Pharmaceutical Basis of Therapeutics*, 6th Edition, MacMillan Publishing Co., New York (1980) can be consulted.

[0076] In some embodiments, the compounds of the present invention can be formulated for parenteral or intravenous administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, *e.g.*, in ampules or in multi-dose containers, with an added preservative. In some embodiments, the compositions of the present invention can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain excipients such as suspending, stabilizing and/or dispersing agents. In some embodiments, a thioredoxin inhibitor such as PX-12, may be supplied in PEG 400 at a unit dosage of about 25 to about 45 mg/mL, and in other embodiments, a thioredoxin inhibitor may be contained in a diluent, for example, HCl. In some embodiments, the thioredoxin inhibitor is diluted in, for example, aqueous polyethylene glycol, at a concentration of from about 1 mg/mL to about 5 mg/mL, prior to administration.

[0077] In some embodiments, the compounds of the present invention can be formulated as a depot preparation. Such long acting formulations can be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. In some embodiments, depot injections can be administered at about 1 month to about 6 month intervals, or longer. For example, in some embodiments the compounds can be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[0078] The compounds of the present invention can also be administered in combination with other active ingredients such as, for example, adjuvants, protease inhibitors, or other compatible drugs or compounds where such combination is seen to be desirable or advantageous in achieving the desired effects of the methods described herein.

[0079] In some embodiments, the disintegrant component comprises one or more of croscarmellose sodium, carmellose calcium, crospovidone, alginic acid, sodium alginate, potassium alginate, calcium alginate, an ion exchange resin, an effervescent system based on food acids and an alkaline carbonate component, clay, talc, starch, pregelatinized starch, sodium starch glycolate, cellulose floc, carboxymethylcellulose, hydroxypropylcellulose, calcium silicate, a metal carbonate, sodium bicarbonate, calcium citrate, or calcium phosphate.

[0080] In some embodiments, the diluent component comprises one or more of mannitol, lactose, sucrose, maltodextrin, sorbitol, xylitol, powdered cellulose, microcrystalline cellulose, carboxymethylcellulose, carboxyethylcellulose, methylcellulose, ethylcellulose, hydroxyethylcellulose, methylhydroxyethylcellulose, starch, sodium starch glycolate, pregelatinized starch, a calcium phosphate, a metal carbonate, a metal oxide, or a metal aluminosilicate.

[0081] In some embodiments, the optional lubricant component, when present, comprises one or more of stearic acid, metallic stearate, sodium stearyl fumarate, fatty acid, fatty alcohol, fatty acid ester, glyceryl behenate, mineral oil, vegetable oil, paraffin, leucine, silica, silicic acid, talc, propylene glycol fatty acid ester, polyethoxylated castor oil, polyethylene glycol, polypropylene glycol, polyalkylene glycol, polyoxyethylene-glycerol fatty ester, polyoxyethylene fatty alcohol ether, polyethoxylated sterol, polyethoxylated castor oil, polyethoxylated vegetable oil, or sodium chloride.

[0082] As used herein, the term "fatty acid", employed alone or in combination with other terms, refers to an aliphatic acid that is saturated or unsaturated. In some embodiments, the fatty acid is a mixture of different fatty acids. In some embodiments, the fatty acid has between about eight to about thirty carbons on average. In some embodiments, the fatty acid has about eight to about twenty-four carbons on average. In some embodiments, the fatty acid has about twelve to about eighteen carbons on average. In some embodiments, suitable fatty acids include, but are not limited to, stearic acid, lauric acid, myristic acid, erucic acid, palmitic acid, palmitoleic acid, capric acid, caprylic acid, oleic acid, linoleic acid, linolenic acid, hydroxystearic acid, 12-hydroxystearic acid, cetostearic acid, isostearic acid, sesquioleic acid, sesqui-9-octadecanoic acid, sesquiisooctadecanoic acid, benhenic acid, isobehenic acid, and arachidonic acid, or mixtures thereof.

[0083] As used herein, the term "fatty acid ester" refers to a compound formed between a fatty acid and a hydroxyl containing compound. In some embodiments, the fatty acid ester is a sugar ester of fatty acid. In some embodiments, the fatty acid ester is a glyceride of fatty acid. In some embodiments, the fatty acid ester is an ethoxylated fatty acid ester.

[0084] As used herein, the term "fatty alcohol", employed alone or in combination with other terms, refers to an aliphatic alcohol that is saturated or unsaturated. In some embodiments, the fatty alcohol is a mixture of different fatty alcohols. In some embodiments, the fatty alcohol

has between about eight to about thirty carbons on average. In some embodiments, the fatty alcohol has about eight to about twenty-four carbons on average. In some embodiments, the fatty alcohol has about twelve to about eighteen carbons on average. In some embodiments, suitable fatty alcohols include, but are not limited to, stearyl alcohol, lauryl alcohol, palmityl alcohol, palmitolyl acid, cetyl alcohol, capryl alcohol, caprylyl alcohol, oleyl alcohol, linolenyl alcohol, arachidonic alcohol, behenyl alcohol, isobehenyl alcohol, selachyl alcohol, chimyl alcohol, and linoleyl alcohol, or mixtures thereof.

[0085] In other embodiments, suitable mannitols include, but are not limited to, PharmMannidex (available from Cargill), Pearlitol (available from Roquette), and Mannogem (available from SPI Polyols).

[0086] As used herein, the term "mineral oil" refers to both unrefined and refined (light) mineral oil. Suitable mineral oils include, but are not limited to, the Avatech™ grades (available from Avatar Corp.), Drakeol™ grades (available from Penreco), Sirius™ grades (available from Shell), and the Citation™ grades (available from Avater Corp.).

[0087] As used herein, the term "polyethoxylated castor oil", refers to a compound formed from the ethoxylation of castor oil, wherein at least one chain of polyethylene glycol is covalently bound to the castor oil. In some embodiments, the castor oil may be hydrogenated or unhydrogenated. Synonyms for polyethoxylated castor oil include, but are not limited to polyoxyl castor oil, hydrogenated polyoxyl castor oil, mcrogolglyceroli ricinoleas, macrogolglyceroli hydroxystearas, polyoxyl 35 castor oil, and polyoxyl 40 hydrogenated castor oil. In some embodiments, suitable polyethoxylated castor oils include, but are not limited to, the Nikkol™ HCO series (available from Nikko Chemicals Co. Ltd.), such as Nikkol HCO-30, HC-40, HC-50, and HC-60 (polyethylene glycol-30 hydrogenated castor oil, polyethylene glycol-40 hydrogenated castor oil, polyethylene glycol-50 hydrogenated castor oil, and polyethylene glycol-60 hydrogenated castor oil, Emulphor™ EL-719 (castor oil 40 mole-ethoxylate, available from Stepan Products), the Cremophore™ series (available from BASF), which includes Cremophore RH40, RH60, and EL35 (polyethylene glycol-40 hydrogenated castor oil, polyethylene glycol-60 hydrogenated castor oil, and polyethylene glycol-35 hydrogenated castor oil, respectively), and the Emulgin® RO and HRE series (available from Cognis PharmaLine). In further embodiments, other suitable polyoxyethylene castor oil

derivatives include those listed in R. C. Rowe and P. J. Shesky, *Handbook of Pharmaceutical Excipients*, (2006), 5th ed., which is incorporated herein by reference in its entirety.

[0088] As used herein, the term "polyethoxylated sterol" refers to a compound, or mixture of compounds, derived from the ethoxylation of sterol molecule. In some embodiments, suitable polyethoyxlated sterols include, but are not limited to, PEG-24 cholesterol ether, Solulan™ C-24 (available from Amerchol); PEG-30 cholestanol, Nikkol™ DHC (available from Nikko); Phytosterol, GENEROL™ series (available from Henkel); PEG-25 phyto sterol, Nikkol™ BPSH-25 (available from Nikko); PEG-5 soya sterol, Nikkol™ BPS-5 (available from Nikko); PEG-10 soya sterol, Nikkol™ BPS-10 (available from Nikko); PEG-20 soya sterol, Nikkol™ BPS-20 (available from Nikko); and PEG-30 soya sterol, Nikkol™ BPS-30 (available from Nikko). As used herein, the term "PEG" refers to polyethylene glycol.

[0089] As used herein, the term "polyethoxylated vegetable oil" refers to a compound, or mixture of compounds, formed from ethoxylation of vegetable oil, wherein at least one chain of polyethylene glycol is covalently bound to the vegetable oil. In some embodiments, the fatty acids has between about twelve carbons to about eighteen carbons. In some embodiments, the amount of ethoxylation can vary from about 2 to about 200, about 5 to 100, about 10 to about 80, about 20 to about 60, or about 12 to about 18 of ethylene glycol repeat units. The vegetable oil may be hydrogenated or unhydrogenated. In some embodiments, suitable polyethoxylated vegetable oils, include but are not limited to, Cremaphor™ EL or RH series (available from BASF), Emulphor™ EL-719 (available from Stepan products), and Emulphor™ EL-620P (available from GAF).

[0090] As used herein, the term "polyethylene glycol" refers to a polymer containing ethylene glycol monomer units of formula $-O-CH_2-CH_2-$. Suitable polyethylene glycols may have a free hydroxyl group at each end of the polymer molecule, or may have one or more hydroxyl groups etherified with a lower alkyl, e.g., a methyl group. Also suitable are derivatives of polyethylene glycols having esterifiable carboxy groups. In some embodiments, polyethylene glycols useful in the present invention can be polymers of any chain length or molecular weight, and can include branching. In some embodiments, the average molecular weight of the polyethylene glycol is from about 200 to about 9000. In some embodiments, the average molecular weight of the polyethylene glycol is from about 200 to about 5000. In some embodiments, the average molecular weight of the polyethylene glycol is from about 200 to

about 900. In some embodiments, the average molecular weight of the polyethylene glycol is about 400. In further embodiments, suitable polyethylene glycols include, but are not limited to polyethylene glycol-200, polyethylene glycol-300, polyethylene glycol-400, polyethylene glycol-600, and polyethylene glycol-900. The number following the dash in the name refers to the average molecular weight of the polymer. In some embodiments, the polyethylene glycol is polyethylene glycol-400. In yet other embodiments, suitable polyethylene glycols include, but are not limited to the Carbowax™ and Carbowax™ Sentry series (available from Dow), the Lipoxol™ series (available from Brenntag), the Lutrol™ series (available from BASF), and the Pluriol™ series (available from BASF).

[0091] As used herein, the term "propylene glycol fatty acid ester" refers to an monoether or diester, or mixtures thereof, formed between propylene glycol or polypropylene glycol and a fatty acid. Fatty acids that are useful for deriving propylene glycol fatty alcohol ethers include, but are not limited to, those defined herein. In some embodiments, the monoester or diester is derived from propylene glycol. In some embodiments, the monoester or diester has about 1 to about 200 oxypropylene units. In some embodiments, the polypropylene glycol portion of the molecule has about 2 to about 100 oxypropylene units. In some embodiments, the monoester or diester has about 4 to about 50 oxypropylene units. In some embodiments, the monoester or diester has about 4 to about 30 oxypropylene units. In some embodiments, suitable propylene glycol fatty acid esters include, but are not limited to, propylene glycol laurates: Lauroglycol™ FCC and 90 (available from Gattefosse); propylene glycol caprylates: Capryol™ PGMC and 90 (available from Gatefosse); and propylene glycol dicaprylocaprates: Labrafac™ PG (available from Gatefosse).

[0092] In some embodiments, suitable sorbitols include, but are not limited to, PharmSorbidex E420 (available from Cargill), Liponic 70-NC and 76-NC (available from Lipo Chemical), Neosorb (available from Roquette), Partech SI (available from Merck), and Sorbogem (available from SPI Polyols).

[0093] Other excipients include starch, sodium starch glycolate, and pregelatinized starch including, but not limited to, those described in R. C. Rowe and P. J. Shesky, *Handbook of Pharmaceutical Excipients*, (2006), 5th ed., which is incorporated herein by reference in its entirety.

[0094] As used herein, the term "stearoyl macrogol glyceride" refers to a polyglycolized glyceride synthesized predominately from stearic acid or from compounds derived predominately from stearic acid, although other fatty acids or compounds derived from other fatty acids may used in the synthesis as well. In some embodiments, suitable stearoyl macrogol glycerides include, but are not limited to, Gelucire® 50/13 (available from Gattefossé).

[0095] As used herein, the term "vegetable oil" refers to naturally occurring or synthetic oils, which may be refined, fractionated or hydrogenated, including triglycerides. In some embodiments, suitable vegetable oils include, but are not limited to castor oil, hydrogenated castor oil, sesame oil, corn oil, peanut oil, olive oil, sunflower oil, safflower oil, soybean oil, benzyl benzoate, sesame oil, cottonseed oil, and palm oil. In other embodiments, suitable vegetable oils include commercially available synthetic oils such as, but not limited to, Miglyol™ 810 and 812 (available from Dynamit Nobel Chicals, Sweden) Neobee™ M5 (available from Drew Chemical Corp.), Alofine™ (available from Jarchem Industries), the Lubritab™ series (available from JRS Pharma), the Sterotex™ (available from Abitec Corp.), Softisan™ 154 (available from Sasol), Croduret™ (available from Croda), Fancol™ (available from the Fanning Corp.), Cutina™ HR (available from Cognis), Simulsol™ (available from CJ Petrow), EmCon™ CO (available from Amisol Co.), Lipvol™ CO, SES, and HS-K (available from Lipo), and Sterotex™ HM (available from Abitec Corp.). In yet other embodiments, suitable vegetable oils, include sesame, castor, corn, and cottonseed oils, and those listed in R. C. Rowe and P. J. Shesky, *Handbook of Pharmaceutical Excipients*, (2006), 5th ed., which is incorporated herein by reference in its entirety.

[0096] This invention and embodiments illustrating the method and materials used may be further understood by reference to the following non-limiting examples.

EXAMPLE 1

[0097] This is a phase Ib, open-label, dose escalation study designed to determine the MTD of PX-12 in patients with advanced or metastatic cancer or malignant lymphoma. A total of up to 28 patients meeting the eligibility criteria were enrolled. Patients were enrolled in cohorts receiving successively higher doses of PX-12 delivered as a 72 hour IV infusion every 21 days. Dose levels of 300, 400, and 500 mg/m^2/day were administered until a dose limiting toxicity (DLT) occurred. Three patients received the starting dose as a 72 hour continuous infusion and were then observed on a 21 day cycle. If all patients in a cohort tolerated the PX-12

dose without unacceptable toxicity, the next dosing cohort was accrued. Advancement of dose cohorts was based upon the degree of drug-related toxicity seen in the first cycle of each dose cohort. When DLT was reached, the previous dose level was determined to be the MTD and the cohort was expanded to include 10 total patients. Patients were allowed to receive additional cycles of PX-12 treatment until documented progressive disease.

[0098] In general, the drug was administered intravenously, via continuous infusion for 72 hours on the first three days of a repeating 21 day cycle. Cohorts of patients were sequentially assigned to receive increasing dose levels of PX-12. Upon determination of eligibility and after registration, patients were assigned to a dose cohort. The appropriate dose of study drug (total mg) was calculated according to the assigned dose level of the cohort and the patient's body surface area (BSA). No dose modifications were permitted during cycle one of treatment. Patients who required dose modification for reasons of toxicity during cycle one were declared to have experienced a DLT. Patients were not allowed to "make up" doses. The sponsor's Medical Monitor was notified in the event of dose modification or delay in cycle two and beyond.

[0099] Patient safety was monitored with periodic physical exams, hematology and chemistry laboratory studies, and assessment of adverse events. Patients in the expansion phase of the trial were eligible to undergo DCE-MRI (dynamic contrast enhanced – magnetic resonance imaging) assessment of microvascular volume and permeability changes pre- and post-study drug treatment. All patients had an initial tumor assessment, an assessment after two cycles of therapy, and an assessment every two cycles thereafter (i.e., after cycles 4, 6, 8, etc.). Blood samples were obtained for pharmacokinetic and pharmacodynamic measures.

EXAMPLE 2

Determination of MTD

[00100] The starting dose level was 300 mg/m^2/day. Dose escalation continued to 400, and 500 mg/m^2/day until MTD was defined. Three patients received the starting dose of 300 mg/m^2/day as a 72 hour continuous infusion and were observed on a 21 day cycle. Advancement of dose cohorts was based upon the degree of drug-related toxicity seen in the first cycle of each dose cohort. When all patients in a cohort tolerated the first cycle of therapy without unacceptable toxicity, the next dosing cohort accrued. When one DLT was identified in the first three patients (1:3), then an additional three patients were added to the same cohort. This

occurred at 400 mg/m^2/day, and the dose cohort was expanded. When no additional DLT was identified in these six patients (1:6), the next dosing cohort started to accrue. At 500 mg/m^2/day one (1) patient experienced two DLTs. Accordingly, the previous dose level (400 mg/m^2/day was defined as MTD. The MTD cohort was expanded to include a total of 10 patients.

EXAMPLE 3

Determination of DLT

[00101] DLT was defined as the occurrence of any of the toxicities listed below except for those that were clearly disease related. Toxicity grades were based on the NCI CTC, Version 3.0. Hematologic: ANC of grade 3 or 4 plus fever, defined as ≥38.5°C (fever had to be present for the grade 3 or 4 ANC to be considered a DLT). ANC of <500/μL for ≥5 days. Platelet count of <25,000/μL. Gastrointestinal: Grade 3 or 4 nausea, vomiting, or diarrhea if persistent despite optimal antiemetic or anti-diarrheal therapy. Any other grade 3 or 4 toxicity. Hepatic: Grade 3 elevation of transaminases if persistent for >7 days. Any other grade 3 or 4 toxicity. Other: Any other drug-related grade 3 or 4 toxicity aside from alopecia. Any adverse event during cycle one that required a dose modification or a delay in the administration of the next dose, or that resulted in a missed dose.

[00102] Patients who experienced a DLT were allowed continue in the study. Such patients were able to resume treatment at the dose level below that at which the DLT occurred after recovery of the toxicity to no more than grade one severity. Patients who required more than two weeks to recover from a DLT were declared to have unacceptable toxicity and were withdrawn from the study.

EXAMPLE 4

Cohort Review and Advancement

[00103] At study initiation, three patients were enrolled and began receiving the 300 mg/m^2/day of PX-12 as a 72 hour continuous infusion. All patients in the cohort were then observed over the entire first 21 day cycle. All three patients in the cohort completed the first 21 day cycle, adverse event (AE) and laboratory data were collected and reviewed by a committee composed of the investigators and sponsor's medical monitor (the Dose Advancement Review Committee). Advancement to the next dose cohort was based upon the degree of drug-related toxicity seen in the first cycle of each new dose cohort. All patients in the cohort tolerated the

PX-12 dose without unacceptable toxicity, and the next dosing cohort was accrued. The result of the Dose Advancement Review Committee review determined whether: 1) the study could advance to the next cohort (including next dose level and date enrollment can begin); and 2) additional patients were needed. Safety results obtained from each cohort were reviewed by the investigators and medical monitor in order to determine whether the next dose cohort could start. At the dose of 500 mg/m^2/day one (1) patient experienced two DLTs and the Dose Advancement Review Committee determined that 400 mg/m^2/day was the MTD. A total of 10 patients were enrolled at this dose level.

EXAMPLE 5

Preparation of PX-12

[00104] PX-12 is currently provided sterile, filtered in PEG 400 (3.5 ml; 36.07 mg/mL) overfilled in a 5 cc glass vial. Sterile 0.013 N aqueous HCl, 46.5 mL (diluent) is delivered in a separate 60 ml vial. PX-12 can be stored frozen or refrigerated between –8 and +4 degrees Celsius.

[00105] The final preparation of product, PX-12 2.5 mg/ml, is carried out at the clinical site with the addition of 3.5 ml PEG 400 containing PX-12 to the 46.5 ml aqueous acid in an aseptic manner, to provide 50 mL of a 2.5 mg/mL solution. This product is non-hemolytic and biocompatible. The appropriate dose of PX-12 is calculated at the clinical site and that volume of drug solution is syringe filtered into D5/W (dextrose injection 5%) to provide a final volume of 250 mL. The prepared PX-12 for infusion can be refrigerated or stored at room temperature and should be used within 36 hours, preferably within 24 hours, of reconstitution.

EXAMPLE 6

Cough and Odor

[00106] PX-12 is rapidly metabolized after administration, providing a major metabolite, 2-butanethiol which has been identified and quantified in expired air of patients. This metabolite produces a garlic like odor at low concentrations, and higher concentrations are pungent and can irritate the respiratory tract. 2-butanethiol is non-cytotoxic at doses of PX-12 which provide biological activity.

[00107] It has been observed that patients experience a mild cough (grade 1) occurring after a few minutes of PX-12 infusion, lasting 10-15 minutes. The cough was most noticeable on

day 1 and appeared to subside on subsequent treatment days. The cough appeared to increase in intensity at doses above 96 mg/m^2/hr. Although PX-12 is odorless, a garlic type pungent odor due to metabolite 2-butanethiol, developed minutes into the infusion of PX-12. The intensity of the odor appeared to increase with higher doses (above 96 mg/m^2), so that air purifiers and a closed ventilation system, were required for administration.

[00108] At the 96 mg/m^2 cohort, the highest administered in the 1 hr infusion due to development of drug-related cough and a pungent odor emitted in the expired patient breath, the protocol was amended to allow for a longer, (3 hour) infusion time. Over the course of the 3 hour infusion, dose escalation was able to reach higher doses than could be achieved in the 1 hr administration and total doses of 226 mg/m^2 and 300 mg/m^2 were administered. At both doses, the expired 2-butanethiol caused temporary eye and throat irritation to patients and staff.

[00109] At the dose of 300 mg/m^2 administered via continuous infusion over 3 hours, one patient developed a reversible pneumonitis on day 5 of cycle 1. This was characterized by fever (102 ^{o}F), cough, hypoxia and bilateral interstitial infiltrates on chest X-ray. The patient was hospitalized and treated with high-dose steroids, and oxygen. The symptoms improved over 48 hours, and the infiltrates resolved. The second of 3 patients treated with PX-12 at this dose (300 mg/m^2/3 hrs) developed shortness of breath after his second cycle and CT scan revealed bilateral pneumonitis. Due to the severity of the respiratory toxicity seen at this dose level, although one occurred in the second cycle, further expansion of this dose level cohort was aborted.

[00110] Subsequently, new trial and protocols were developed to expand the time period over which PX-12 was administered via continuous infusion. Under this protocol, PX-12 was administered either as a 24-hr infusion every 7 to 14 days starting at 150 mg/m^2/day (n=18); or as a 72-hr infusion every 21 days, starting at 300 mg/m^2/day (n=14) using a portable delivery pump.

[00111] Both the 24 hour and 72 hour infusions of PX-12 were well tolerated in patients at doses up to 400 mg/m^2/day. Common grade 1/2 AE's included fatigue, taste alteration, and odor caused by expired drug metabolite. In the 24 h study the maximal dose evaluated was 450 mg/m^2. No DLTs were observed in the 24 hour infusion cohort.

[00112] The 24 hour infusion study was then stopped, and a new 72 hour infusion study to evaluate and even longer exposure period. DLT in the 72 hour study at the highest dose administered (500 mg/m^2/day) included reversible hypoxia with or without pneumonitis.

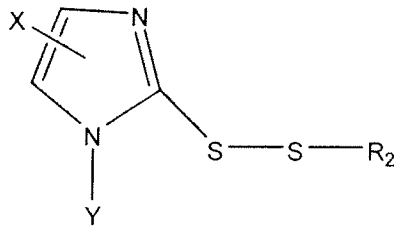
[00113] Best response in these two studies was stable disease in 3 pts (SD): liver cancer and colon cancer (24 hour infusion) and rectal cancer (72 hour infusion). PX-12 lowered circulating Trx-1 levels in patients who had starting Trx-1 levels 3-fold greater than that of the normal population (5.4 ng/mL) including all three SD. Circulating VEGF and FGF-2 levels were also lowered over multiple courses of treatment in these patients. The pharmacokinetics of PX-12 showed a dose dependent increase of C_{max} and no accumulation over multiple cycles following a 24 hr infusion.

[00114] Doses of at least 400 mg/m^2/24hrs appear to be safe and tolerable over 24 hour or 72 hour infusion. However, extending the infusion time to 72 hours decreased the intensity of cough and odor compared to bolus 1-3 hr infusions.

[00115] Although the present invention has been described in considerable detail with reference to certain preferred embodiments thereof, other versions are possible. Therefore the spirit and scope of the appended claims should not be limited to the description and the preferred versions contained within this specification.

J. CLAIMS

1. A method for treating cancer comprising administering a therapeutically effective amount of a compound of formula II:



II

wherein:

R_2 is C_1-C_6 alkyl or arylalkyl;

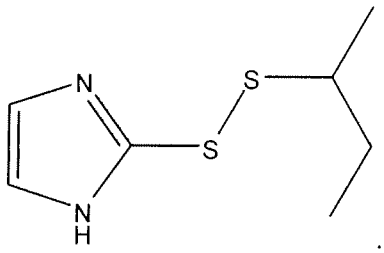
X is H, halo, alkyl, alkoxy, or amino; and

Y is H, alkyl, hydroxyalkyl, or arylalkyl;

via continuous infusion over from about 48 to about 72 hours.

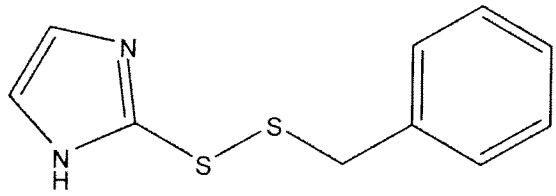
2. The method of claim 1, wherein the continuous infusion is over about 72 hours.

3. The method of claim 1, wherein the compound of formula II is a compound of formula II(a):



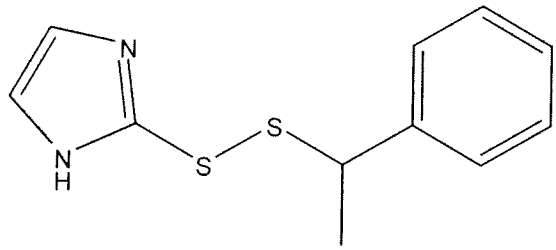
II(a)

4. The method of claim 1, wherein the compound of formula II is a compound of formula II(b):



II(b)

5. The method of claim 1, wherein the compound of formula II is a compound of formula II(c):



II(c)

6. The method of claim 1, wherein said continuous infusion is repeated about every fourteen to about every twenty-eight days.

7. The method of claim 4, wherein said continuous infusion is repeated about every twenty-one days.

8. The method of claim 1, wherein said therapeutically effective amount of a compound of formula I is from about 300 mg/m^2 to about 3000 mg/m^2.

9. The method of claim 2, wherein said therapeutically effective amount of a compound of formula I is from about 300 mg/m^2 to about 3000 mg/m^2.

10. The method of claim 9, wherein said therapeutically effective amount of a compound of formula I is from about 600 mg/m^2 to about 2100 mg/m^2.

11. The method of claim 10, wherein said therapeutically effective amount of a compound of formula I is from about 900 mg/m^2 to about 1800 mg/m^2.

12. The method of claim 11, wherein said therapeutically effective amount of a compound of formula I is about 900 mg/m^2.

13. The method of claim 11, wherein said therapeutically effective amount of a compound of formula I is about 1200 mg/m^2.

14. The method of claim 13, wherein said therapeutically effective amount of a compound of formula I is administered via continuous infusion at a rate of about 400 mg/m^2/24 hours.

15. The method of claim 11, wherein said therapeutically effective amount of a compound of formula I is about 1500 mg/m^2.

16. The method of claim 1, wherein said cancer is metastatic cancer.

17. The method of claim 1, wherein said cancer is selected from the group consisting of: breast cancer; cervical cancer; colorectal cancer; gastric cancer; hepatoma; hepatocellular cancer; gastrointestinal cancer; liver cancer; lung cancer; myeloma; non-small cell lung cancer; ovarian cancer; pancreatic cancer; prostate cancer; renal cancer; small-cell lung cancer; and squamous cell cancer.

18. The method of claim 1, further comprising the administration of a therapeutically effective amount of a secondary agent.

19. The method of claim 18, wherein the secondary agent is a chemotherapeutic agent.

20. The method of claim 19, wherein said chemotherapeutic agent is selected from the group consisting of: cisplatin; tamoxifen; and paclitaxel.

21. The method of claim 18, wherein the secondary agent is an antithrombotic.

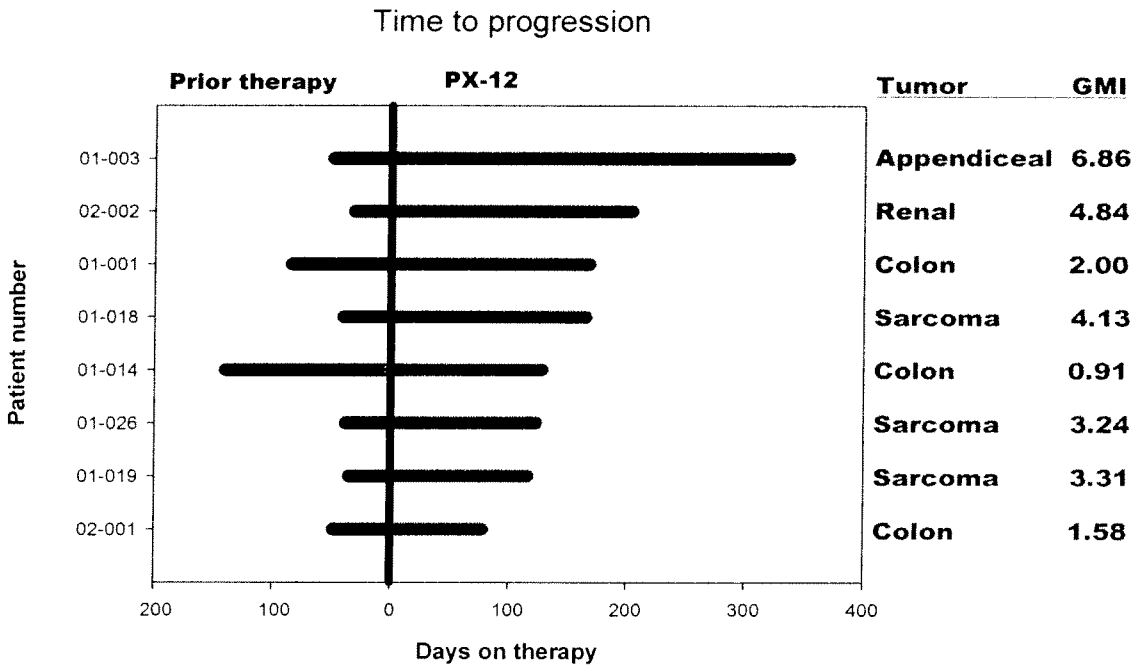

Time to progression
Prior therapy
PX-12
Tumor
GMI
Patient number
01-003
02-002
01-001
01-018
01-014
01-026
01-019
02-001
Appendiceal 6.86
Renal 4.84
Colon 2.00
Sarcoma 4.13
Colon 0.91
Sarcoma 3.24
Sarcoma 3.31
Colon 1.58
200
100
0
100
200
300
400
Days on therapy

FIGURE 1

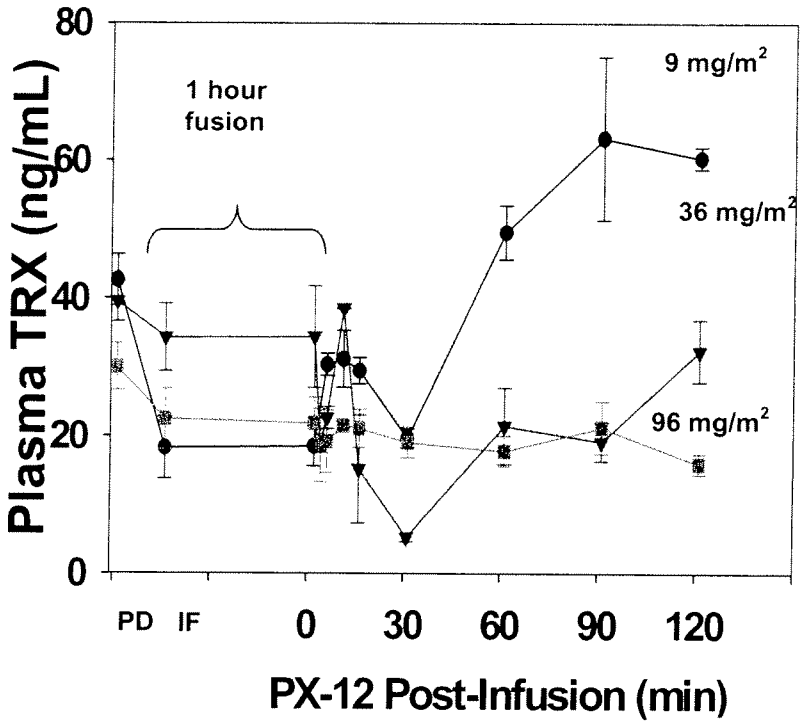

80
60
40
20
0
Plasma TRX (ng/mL)
1 hour
fusion
9 mg/m2
36 mg/m2
96 mg/m2
PD
IF
0
30
60
90
120
PX-12 Post-Infusion (min)

FIGURE 2

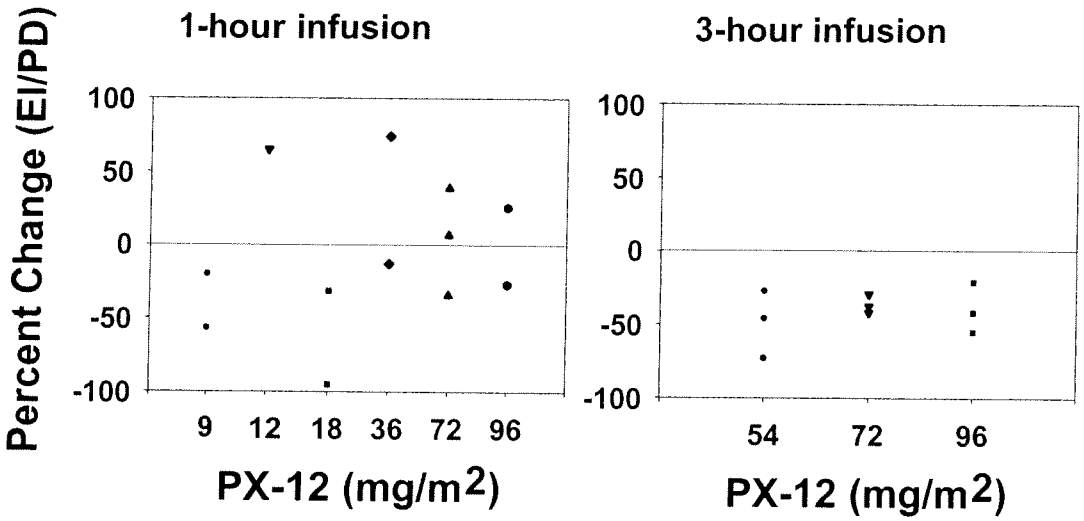

1-hour infusion
3-hour infusion
Percent Change (EI/PD)
100
50
0
-50
-100
9
12
18
36
72
96
54
72
96
PX-12 (mg/m2)
PX-12 (mg/m2)

FIGURE 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 08/87623

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 57/00 (2009.01)
USPC - 514/128; 514/47,128;536/27.3
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A01N 57/00 (2009.01)
USPC: 514/128

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

USPC: 514/47,128;536/27.3

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Pubwest, Google, Google scholar (ASYMMETRIC DISULFIDES, thioredoxin inhibitor, 1-methylpropyl 2-imidazoly1 disulfide (PX-12), metastatic cancer, cancer, continuous infusion, hour, day, secondary agent, chemotherapeutic agent, cisplatin; tamoxifen; and paclitaxel, antithrombotic)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	Ramanathan et al , A Phase I Pharmacokinetic and Pharmacodynamic Study of PX-12, a Novel Inhibitor of Thioredoxin-1, in Patients with Advanced Solid Tumors, Clin Cancer Res 2109 2007;13(7) April 1, 2007 abstract	1, 2, 3, 6- 9 ---------- 4, 5, 10-21
Y	US 2004/0116496 A1 (KIRKPATRICK et al) 17 June 2004 (17.06.2004) para [0038]-[0103]	4, 5, 10-19
Y	US 6,730,064 B2 (RAGHEB et al) 4 May 2004 (04.05.2004) col 3 and col 9	20-21

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
06 Feb. 2009 (06.02.2009)	24 FEB 2009

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (April 2007)