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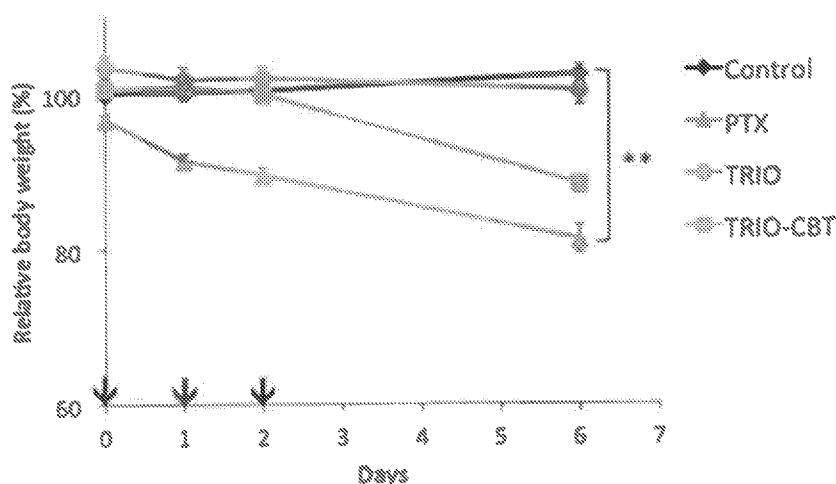
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(54) Title: MULTI-DRUG MICELLE COMBINATIONS WITH CARBOPLATIN

FIG. 1



(57) Abstract: Provided are compositions, kits, and methods of treatment including administering to lung, breast, gynecological, and/or gastrointestinal cancer cells a therapeutically effective amount of carboplatin in conjunction with a micelle composition that includes a therapeutically effective amount of paclitaxel, 17-AAG, and rapamycin, wherein the weight ratio of paclitaxel, 17-AAG, rapamycin, and carboplatin is about 1:1:1 to about 5:5:1:10 and the micelle includes poly(ethylene glycol)-block-poly(lactic acid) (PEG-b-PLA).



MULTI-DRUG MICELLE COMBINATIONS WITH CARBOPLATIN

STATEMENT OF GOVERNMENT SUPPORT

[0001] This invention was made with government support under AI101157 awarded by the National Institutes of Health. The United States government has certain rights in the invention.

FIELD

[0002] The present technology relates generally to treatment of lung cancer, breast cancer, gynecological cancer, and gastrointestinal cancer with a combination carboplatin (CBT) and a micelle loaded with paclitaxel (PTX), 17-allylamino-17-dimethoxy-geldanamycin (17-AAG), and rapamycin (RAPA).

SUMMARY

[0003] The present technology provides methods for the treatment of lung cancer, breast cancer, gynecological cancer, and gastrointestinal cancer employing a combination of carboplatin and a multi-drug micelle chemotherapy including paclitaxel, 17-AAG, and rapamycin. The weight ratio of paclitaxel, 17-AAG, rapamycin, and carboplatin may be about 1:1:1:1 to about 5:5:1:10. It has been found that carboplatin in combination with the micelles exhibit synergistic anti-cancer activity *in vitro* and/or potent anti-cancer efficacy *in vivo*. The micelles include poly(ethylene glycol)-block-poly(lactic acid) (PEG-b-PLA) and are highly effective at solubilizing the hydrophobic anti-cancer drugs. The method may include administering a composition that includes the carboplatin and the micelle composition. Various lung cancers may be treated by the present methods, including small cell lung cancer and/or non-small cell lung cancer (*e.g.*, squamous cell carcinoma, adenocarcinoma, and/or large cell carcinoma). In some embodiments, the lung cancer may include non-small cell adenocarcinoma lung cancer). In addition, breast cancer, gynecological cancer, and gastrointestinal cancer may be treated using the present methods.

[0004] The present methods include administering to lung cancer, breast cancer, gynecological cancer, and gastrointestinal cancer, or a combination of two or more thereof, a therapeutically effective amount of carboplatin and a therapeutically effective amount of a micelle composition including paclitaxel, 17-AAG, and rapamycin. The weight ratio of paclitaxel, 17-AAG, rapamycin and carboplatin, may be about 1:1:1:1 to about 5:5:1:10. For example, the composition may have a ratio by mass of about 1:1:1:1 to about 5:5:1:8 of paclitaxel, 17-AAG,

rapamycin, and carboplatin or about 2:2:1:2 to about 2:3:1:5. In any embodiment of the present methods, the therapeutically effective amount of each drug may be about 2-90 mg/kg of paclitaxel, about 2-90 mg/kg of 17-AAG, about 1-50 mg/kg of rapamycin, and about 2-90 mg/kg of carboplatin. The micelles include PEG-b-PLA and contain paclitaxel, 17-AAG, and rapamycin. The poly(ethylene glycol) block of the PEG-b-PLA may have a molecular weight (M_n) of about 1,500 g/mol to about 30,000 g/mol, and the poly(lactic acid) block of the PEG-b-PLA may have a molecular weight of about 1,500 to about 30,000 g/mol.

[0005] The present methods also include administration to the cancer cells *in vitro* or *in vivo*, where *in vivo* administration can be to lung (and/or breast cancer and/or gynecological and/or gastrointestinal) cancer cells in a subject, for example, a human subject.

[0006] The present technology also provides kits and compositions including the carboplatin and the micelle composition described herein.

[0007] The foregoing summary is illustrative only and is not intended to be in any way limiting. In addition to the illustrative aspects, embodiments and features described above, further aspects, embodiments and features will become apparent by reference to the following drawings and the detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] FIG. 1 shows acute toxicity after daily IV injection for 3 days (Day 0, 1 and 2) in healthy BALB/c mice: Control (Saline), PTX (PTX-loaded micelle at 50 mg/kg), TRIO (PTX, 17-AAG, and RAPA (60, 60, and 30 mg/kg)) and TRIO-CBT (PTX, 17-AAG, and RAPA (60, 60, and 30 mg/kg) and 60 mg/kg CBT) by tail-vein IV injections. The results of relative body weight (%) are shown as Mean $\pm$ SD, n = 3 per group, **, $P < 0.05$.

[0009] FIGS. 2A-2C show antitumor activity of PTX-CBT, TRIO, and TRIO-CBT in an A549 tumor xenograft mouse model. Mice bearing A549 flank tumors were treated with Control (saline), PTX-CBT (PTX and CBT at 15 and 15 mg/kg), TRIO (PTX, 17-AAG, and RAPA (15, 15, and 7.5 mg/kg)) and TRIO-CBT (PTX, 17-AAG, and RAPA (15, 15, and 7.5 mg/kg) and 15 mg/kg CBT) by tail vein IV injection. FIG. 2A show tumor volume, FIG. 2B shows Kaplan-Meier survival analysis, and FIG. 2C shows relative body weight. Mice received 3 weekly injections followed by one week off for 3 cycles. Mean $\pm$ SD, n = 6 per group, **, $P < 0.05$.

DETAILED DESCRIPTION

[0010] In the following detailed description, reference is made to the accompanying drawings, which form a part hereof. In the drawings, similar symbols typically identify similar components, unless context dictates otherwise. The illustrative embodiments described in the detailed description, drawings, and claims are not meant to be limiting. Other embodiments may be utilized, and other changes may be made, without departing from the spirit or scope of the subject matter presented here.

[0011] All publications, patent applications, issued patents, and other documents referred to in this specification are herein incorporated by reference as if each individual publication, patent application, issued patent, or other document was specifically and individually indicated to be incorporated by reference in its entirety. Definitions that are contained in text incorporated by reference are excluded to the extent that they contradict definitions in this disclosure.

[0012] The present technology provides new methods of treating lung, breast, gynecological, and/or gastrointestinal cancer that take advantage of the surprising effect of combining carboplatin and multi-drug combinations of anti-cancer agents delivered by certain types of micelles. Poly(ethylene glycol)-block-poly(lactic acid) (PEG-b-PLA) forms a micellar multi-drug delivery system, that is advantageous for, but not limited to, poorly water soluble drugs. For example, PEG-b-PLA can safely deliver the three drug combination of paclitaxel, 17-AAG, and rapamycin in cancer treatments (*e.g.*, Triolimus or TRIO, which contains all three drugs). It has now been discovered that combinations of carboplatin in conjunction with TRIO provide significantly enhanced anti-cancer effects compared to the combination therapy of carboplatin and paclitaxel alone. Certain combinations of paclitaxel, 17-AAG, rapamycin, and carboplatin may not only slow tumor growth but drive tumor regression. In some embodiments, the present technology may also have reduced toxicity compared to the combination therapy of carboplatin and paclitaxel alone.

[0013] Thus, the present technology provides methods of treating cancer (*e.g.*, lung cancer) or inhibiting cancer cell (*e.g.*, lung cancer cell) proliferation that include administering a therapeutically effective amount of carboplatin in conjunction with a micelle composition comprising a therapeutically effective amount of paclitaxel, 17-AAG, and rapamycin, wherein the cancer cells include lung, breast, gynecological, gastrointestinal cancer cells, or a combination of two or more thereof; the weight ratio of paclitaxel, 17-AAG, rapamycin, and

carboplatin is about 1:1:1:1 to about 5:5:1:10; and the micelle composition includes poly(ethylene glycol)-block-poly(lactic acid).

[0014] The present technology also provides a therapeutically effective amount of carboplatin in conjunction with the micelle compositions described herein for use in a method of treating lung, breast, gynecological, gastrointestinal cancer, or a combination of two or more thereof. In some embodiments, the method includes administering the therapeutically effective amount of carboplatin and the micelle composition to lung, breast, gynecological, gastrointestinal cancer cells, or a combination of two or more thereof.

[0015] In some aspects, the present methods can include administering to lung, breast, gynecological, gastrointestinal cancer cells, or a combination of two or more thereof, a therapeutically effective amount of carboplatin in conjunction with the micelle compositions described herein *in vitro*. For example, the present methods can include administering to lung, breast, gynecological, or gastrointestinal cancer cells harvested from laboratory culture, or any similar *in vitro* cell growth procedure known to a person having ordinary skill in the art, a therapeutically effective amount of carboplatin in conjunction with paclitaxel, 17-AAG, and rapamycin-loaded micelle.

[0016] In some aspects, the present methods include administering to lung, breast, gynecological, or gastrointestinal cancer cells a therapeutically effective amount of carboplatin in conjunction with the micelle composition described herein *in vivo*. In some embodiments, the lung, breast, gynecological, or gastrointestinal cancer cells are in a subject, such as a human or animal subject (e.g., mouse, rat, dog, cat, pig, horse, cow, monkey or the like). In some aspects, the present methods include administering to a subject having lung, breast, gynecological, gastrointestinal cancer, or a combination of two or more thereof, a therapeutically effective amount of carboplatin and a therapeutically effective amount of the micelle compositions as described herein. In certain embodiments, the micelle composition comprises an aqueous carrier and is administered to the subject intravenously or intraperitoneally.

[0017] In some embodiments, the methods inhibit cancer cell growth at least about 100 days after administration. In some embodiments, the methods inhibit cancer cell growth at least about 110 days, at least about 120 days, at least about 130 days, or at least 140 days after administration.

[0018] Three-drug micelle compositions of rapamycin, paclitaxel, and 17-AAG can be prepared using amphiphilic diblock polymers such as PEG-b-PLA as set forth, *e.g.*, in US 8,858,965 (incorporated by reference in its entirety herein). While not wishing to be bound by theory, it is believed that the hydrophobic poly(lactic acid) block of the polymers orient toward the interior of each micelle, and the hydrophilic poly(ethylene glycol) block of the polymers orient toward the exterior of each micelle. The drugs described herein are non-covalently associated with the micelles such that the drugs are solubilized by the micelles, thereby forming drug delivery systems. For example, the drugs described herein may be encapsulated by the micelles, and/or present in and/or on the micelle membrane.

[0019] In the present technology, each of the polymer blocks of the PEG-b-PLA which forms the micelles, may encompass a range of molecular weights so long as the resulting polymer is capable of forming micelles in aqueous solution. For example, the molecular weight of the poly(ethylene glycol) block can be about 1,500 to about 30,000 g/mol, and the molecular weight of the poly(lactic acid) block can be about 1,500 to about 30,000 g/mol. Suitable molecular weights for the poly(ethylene glycol) block include about 1,500, about 2,000, about 3,000, about 4,000, about 5,000, about 6,000, about 8,000, about 10,000, about 12,000, about 14,000, about 20,000, about 25,000, or about 30,000 g/mol, or a range between and including any two of the foregoing values. Suitable molecular weights for the poly(lactic acid) block include about 1,500, about 2,000, about 3,000, about 4,000, about 5,000, about 6,000, about 7,000, about 10,000, about 12,000, about 14,000, about 20,000, about 25,000, or about 30,000 g/mol, or a range between and including any two of the foregoing values. In some embodiments of the present technology, the molecular weight of the poly(ethylene glycol) block can be about 4,000 to about 10,000 g/mol, and the molecular weight of the poly(lactic acid) block can be about 2,000 to about 5,000 g/mol. In certain embodiments, the molecular weight of the poly(ethylene glycol) block can be about 10,000 to about 14,000 g/mol, and the molecular weight of the poly(lactic acid) block can be about 5,000 to about 7,000 g/mol. In any embodiment, the poly(ethylene glycol) block of the PEG-b-PLA may have a molecular weight (M_n) of about 1,750 g/mol to about 15,000 g/mol, about 2,000 g/mol to about 10,000 g/mol, or about 3,000 g/mol to about 5,000 g/mol; and the poly(lactic acid) block of the PEG-b-PLA may have a molecular weight of about 1,500 to about 20,000 g/mol, about 1,750 to about 10,000 g/mol, or about 2,000 to about 5,000 g/mol. Examples of suitable combinations of molecular weights for PEG-b-PLA include, but are not limited to, about 2,000-2,000 (2K-2K), about 3K-5K, about 5K-

3K, about 5K-6K, about 6K-5K, about 6K-6K, about 8K-4K, about 4K-7K, about 7K-3K, about 3K-7K, about 12K-6K, and/or about 6K-7K g/mol.

[0020] The combined drug loading of micelles used in the present technology can be about 5 wt. % to about 50 wt.% with respect to the weight of the micelles (*i.e.*, wt.% is equal to (combined weight of the drugs)/(wt of the polymers) $\times$ 100). Examples of such combined drug loadings include about 5 wt%, about 10 wt%, about 15 wt%, about 20 wt%, about 25 wt%, about 30 wt%, about 35 wt%, about 40 wt%, about 45 wt%, about 50 wt%, or a range between and including any of the foregoing individual values. Thus, in some embodiments, the combined drug loading is about 5 wt. % to about 40 wt. %, about 5 wt. % to about 30 wt. %, about 10 wt% to about 40 wt%, about 10 wt% to about 30 wt%, about 10 wt. % to about 20 wt. %, or about 20 wt% to about 40 wt%.

[0021] Subject to the above combined drug loading limits, the drug loading in the micelles for each individual drug can be about 1 wt % to about 25 wt %, with respect to the weight of the micelles. The drug loading of each drug can also be about 1.5 wt. % to about 24 wt %, about 1.75 wt % to about 20 wt %, or about 2 wt % to about 15 wt %, with respect to the weight of the micelles. Examples of individual drug loadings include about 1 wt%, about 2 wt%, about 3 wt%, about 4 wt%, about 5 wt%, about 6 wt%, about 8 wt%, about 10 wt%, about 15wt%, about 20 wt%, about 21 wt%, about 22 wt%, about 23 wt%, about 24 wt% or about 25 wt%, or a range between and including any two of the foregoing values.

[0022] The micelles may have a drug loading efficiency of one or more of paclitaxel, 17-AAG, and rapamycin of at least about 70%, at least about 80%, at least about 85%, at least about 90%, or at least about 95%. In some embodiments, the micelles may have a drug loading efficiency of one or more of paclitaxel, 17-AAG, and rapamycin between about 70% and about 100%, about 80% and about 99.99%, about 85% and about 99.9%, or about 90% and about 99%.

[0023] The weight ratio of paclitaxel, 17-AAG, and rapamycin in the micelles of the present technology may range from about 1:1:1 to about 5:5:1, about 1:1:1 to about 5:5:1, about 3:3:2 to about 9:9:2, about 7:7:4 to about 4:4:1, or about 7:7:4 to about 3:4:1. In some embodiments, the weight ratio of paclitaxel, 17-AAG, and rapamycin is about 2:2:1 to about 2:3:1.

[0024] The weight ratio of paclitaxel, 17-AAG, rapamycin, and carboplatin in the present technology may range from about 1:1:1:1 to about 5:5:1:10, about 1:1:1:1 to about 5:5:1:8, about 3:3:2:3 to about 9:9:2:15, about 7:7:4:7 to about 4:4:1:7, or about 7:7:4:7 to about 3:4:1:6. In some embodiments, the weight ratio of paclitaxel, 17-AAG, rapamycin, and carboplatin is about 2:2:1:2 to about 2:3:1:5.

[0025] A therapeutically effective amount of paclitaxel and 17-AAG each individually may be about 1 mg/kg to about 100 mg/kg (drug wt/subject wt) or about 2 mg/kg to about 90 mg/kg. In some embodiments, the therapeutically effective amount of paclitaxel and 17-AAG each individually may be about 30 mg/kg to about 80 mg/kg, about 40 mg/kg to about 75 mg/kg, or about 50 mg/kg to about 70 mg/kg. In some embodiments, the therapeutically effective amount of paclitaxel and 17-AAG each individually may be about 5 mg/kg to about 30 mg/kg, about 10 mg/kg to about 25 mg/kg, or about 10 mg/kg to about 20 mg/kg.

[0026] A therapeutically effective amount of rapamycin may be about 1 mg/kg to about 50 mg/kg (drug wt/subject wt). In some embodiments, the therapeutically effective amount of rapamycin may be about 10 mg/kg to about 50 mg/kg, about 20 mg/kg to about 40 mg/kg, or 25 mg/kg to about 35 mg/kg. In some embodiments, the therapeutically effective amount of rapamycin may be about 2.5 mg/kg to about 20 mg/kg, about 3 mg/kg to about 15 mg/kg, or about 5 mg/kg to about 10 mg/kg.

[0027] A therapeutically effective amount of carboplatin may be about 1 mg/kg to about 100 mg/kg (drug wt/subject wt) or about 2 mg/kg to about 90 mg/kg. In some embodiments, the therapeutically effective amount of carboplatin may be about 30 mg/kg to about 80 mg/kg, about 40 mg/kg to about 75 mg/kg, or about 50 mg/kg to about 70 mg/kg. In some embodiments, the therapeutically effective amount of carboplatin may be about 5 mg/kg to about 30 mg/kg, about 10 mg/kg to about 25 mg/kg, or about 10 mg/kg to about 20 mg/kg.

[0028] The drugs may be delivered in amounts according to drug ratios above. For example, a composition may be prepared containing paclitaxel, 17-AAG, rapamycin, and carboplatin at a drug ratio of about 2:2:1:2 to about 2:3:1:5, and having about 2 to about 90 mg/kg of carboplatin and a micelle composition prepared to deliver about 2 to about 90 mg/kg of paclitaxel, about 2 to about 90 mg/kg of 17-AAG, and about 1 to about 50 mg/kg of rapamycin in the prescribed ratio. Examples of suitable amounts of drugs to be delivered having a ratio ranging from about 2:2:1:2 to about 2:3:1:5 by mass include, but are not limited to, about 2/2/1/2

mg/kg of paclitaxel/17-AAG/rapamycin/carboplatin, about 15/15/7.5/15 mg/kg of paclitaxel/17-AAG/rapamycin/carboplatin, about 30/30/15/30 mg/kg of paclitaxel/17-AAG/rapamycin, about 50/50/25/50 mg/kg of paclitaxel/17-AAG/rapamycin/carboplatin, about 60/60/30/60 mg/kg of paclitaxel/17-AAG/rapamycin/carboplatin, about 70/70/35/70 mg/kg of paclitaxel/17-AAG/rapamycin/carboplatin, about 2/3/1/5 mg/kg of paclitaxel/17-AAG/rapamycin/carboplatin, about 7.5/11.25/3.75/18.75 mg/kg of paclitaxel/17-AAG/rapamycin/carboplatin, about 15/22.5/7.5/37.5 mg/kg of paclitaxel/17-AAG/rapamycin/carboplatin, about 22.5/33.75/11.25/56.25 mg/kg of paclitaxel/17-AAG/rapamycin/carboplatin, about 30/45/15/75 mg/kg of paclitaxel/17-AAG/rapamycin, or a range between and/or including any of the foregoing drug ratios and amounts.

[0029] Paclitaxel, 17-AAG, and rapamycin can be incorporated together into individual PEG-PLA micelles, thereby forming multiple drug micelles (MDM). Alternatively, the drugs can be incorporated individually into PEG-PLA micelles, thereby forming single drug micelles (SDM). Single drug micelles that contain different drugs can then be combined to provide a single drug micelle drug combination (SDMDC) composition, and the micelles can be combined in a single aqueous vehicle to provide a micelle composition.

[0030] The micelle composition may include an aqueous vehicle, wherein the concentration of the drugs is about 0.4 mg/mL to about 25 mg/mL, about 0.6 mg/mL to about 20 mg/mL, about 0.8 mg/mL to about 15 mg/mL, or about 1 mg/mL to about 5 mg/mL, with respect to the volume of the aqueous vehicle. The encapsulated paclitaxel, 17-AAG, and rapamycin can have an aqueous solubility of about 1 mg/mL to about 10 mg/mL when contacted with an aqueous environment.

[0031] Commonly, the composition may include an aqueous carrier. In some embodiments, the composition may have a concentration of the drugs of about 0.4 mg/mL to about 25 mg/mL, about 0.6 mg/mL to about 20 mg/mL, or about 1 mg/mL to about 15 mg/mL, with respect to the volume of the aqueous vehicle. In some embodiments, the aqueous carrier may include water, saline, aqueous carbohydrate solutions and the like. For example, 0.9 % NaCl solution or a 5% aqueous saccharide solution. The aqueous saccharide solution can be, for example, dextrose or glucose. The aqueous carrier can also be any sterile aqueous solutions of water-soluble salts, for example, NaCl. The aqueous solutions can also be isotonic. The aqueous solutions may be suitably buffered. Aqueous solutions, described herein, can be suitable for intravenous, intramuscular, subcutaneous, intraperitoneal, and intratumoral injection. Appropriate sterile

aqueous media can be purchased or can be prepared by standard techniques well known to those skilled in the art.

[0032] The micelle composition can be substantially free of pharmaceutically acceptable organic solvents, including but not limited to, ethanol, dimethyl sulfoxide, castor oil, and castor oil derivatives. For example, the micelle composition can comprise less than about 2 wt. %, less than about 1 wt. %, less than about 0.5 wt. %, or less than about 0.1 wt. %, of organic solvents such as, but not limited to, ethanol, dimethyl sulfoxide, castor oil, and castor oil derivatives, individually or in combination. In some embodiments, the micelle compositions are wholly free of organic solvent.

[0033] The present technology also provides compositions including carboplatin and the micelle compositions as described herein. The methods of the present technology may include administering the composition that includes both the micelle composition and the carboplatin. In some embodiments, the composition may administered intravenously or intraperitoneally.

[0034] The present technology provides a kit including carboplatin and the micelle compositions as described herein. The kit may include a first container (or package) and a second container (or package), wherein the first container includes the carboplatin and the second container includes the micelle composition. In some embodiments, the first container and/or the second container may include an aqueous carrier. In another embodiment, an aqueous carrier may be added to the first container and/or the second container. In some embodiments, the first container and the second container may be added together prior to administration.

[0035] Specific dosages of a therapeutically effective amount of carboplatin and a therapeutically effective amount of drug-loaded micelle composition in the present methods may be adjusted depending on conditions of disease, the age, body weight, general health conditions, sex, and diet of the subject, dose intervals, administration routes, excretion rate, and combinations of drugs. Any of the above dosage forms containing effective amounts are well within the bounds of routine experimentation and therefore, well within the scope of the present technology.

DEFINITIONS

[0036] The following terms are used throughout as defined below.

[0037] The term “and/or” means any one of the items, any combination of the items, or all of the items with which this term is associated.

[0038] As used herein, singular articles such as “a”, “an”, and “one” are intended to refer to singular or plural. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as “solely,” “only,” and the like in connection with the recitation of claim elements, or use of a “negative” limitation.

[0039] The term “about” can refer to a variation of up to $\pm 10\%$ of the value specified. For example, “about 50” can in some embodiments carry a variation from 45 to 55 percent. In other embodiments, “about” can refer to a variation of $\pm 1\%$, $\pm 2\%$, or $\pm 5\%$. Unless indicated otherwise herein, the term “about” is intended to include values, *e.g.*, weight percentages, proximate to the recited range that are equivalent in terms of the functionality of the individual ingredient, the composition, or the embodiment. In addition, unless indicated otherwise herein, a recited range (*e.g.*, weight percentages) includes each specific value, integer, decimal, or identity within the range.

[0040] “Treating” or “treatment” within the context of the present technology means an alleviation, in whole or in part, of symptoms associated with a disorder or disease, or slowing, or halting of further progression or worsening of those symptoms. As a non-limiting example of treatment, a subject can be successfully treated for lung cancer if, after receiving through administration a therapeutically effective or therapeutically effective amount of one or more compositions described herein, the subject shows observable and/or measurable reduction in or absence of one or more signs and symptoms of lung cancer such as, but not limited to, reduced tumor size (*e.g.*, reduced tumor volume), reduced morbidity and mortality, or improvement in quality of life relating thereto. Treatment, as defined herein, of a mammal, including a human being, is subject to medical aid with the object of improving the mammal's condition, directly or indirectly. Treatment typically refers to the administration of a therapeutically effective amount of carboplatin and a therapeutically effective amount of a drug-loaded micelle composition as described herein.

[0041] The “administration” of a therapeutically effective amount of carboplatin and a therapeutically effective amount of drug-loaded micelle composition includes, for example, parenteral or topical administration. Parenteral, for example, can be by infusion, injection, such as intravenous, and the patient can be a mammal, for example, a human. Parenteral or systemic

administration includes, but is not limited to, subcutaneous, intravenous, intraperitoneal, and intramuscular injections. Injectable dosage forms of the present methods generally include, but are not limited to, aqueous suspensions, such as aqueous suspensions described herein. Topical administration generally includes, but is not limited to, powders, sprays, ointments, pastes, creams, lotions, gels, solutions, and patches. For example, aerosol sprays can be prepared using compositions described herein, oleic acid, trichloromonofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethane, and a standard, metered dose aerosol dispenser. Upon administration, the composition including the intact micelles can circulate followed by the micelles dissociating into individual polymer chains, and release active agents (*i.e.*, paclitaxel, rapamycin, and 17-AAG) (either by diffusion or micelle composition dissociation), distribute active agents (*i.e.*, paclitaxel, rapamycin, 17-AAG, and carboplatin) into tissue (*e.g.* tumors), and/or undergo renal clearance. The schedule of these events cannot be predicted with specificity, and these events significantly influence the anti-tumor activity of the active agents, such as paclitaxel, rapamycin, 17-AAG, or carboplatin.

[0042] “Effective amount” refers to the amount of a compound or composition required to produce a desired effect. Hence, a “therapeutically effective amount” of a compound or composition of the present technology in the context of treatment refers to an amount of the compound or composition that alleviates, in whole or in part, symptoms associated with a disorder or disease, or slows or halts further progression or worsening of those symptoms. In the context of prevention, a therapeutically effective amount prevents or provides prophylaxis for the disease or disorder in a subject at risk for developing the disease or disorder. Determining a therapeutically effective amount of a compound described herein for treating a particular disorder or disease is well within the skill in the art in view of the present disclosure. In the case of lung cancer, the therapeutically effective amount of the compound or composition may reduce the number of lung cancer cells; reduce the tumor size; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the disorder; inhibit (*i.e.*, slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis. For the treatment of tumor dormancy or micrometastases, the therapeutically effective amount of the compound or composition may reduce the number or proliferation of micrometastases; reduce or prevent the growth of a dormant tumor; or reduce or prevent the recurrence of a tumor after treatment or removal (*e.g.*, using an anti-cancer therapy such as surgery, radiation therapy, or chemotherapy). To the extent the compound or composition may prevent growth and/or kill

existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy *in vivo* can, for example, be measured by assessing the duration of survival, disease free survival (DFS), time to disease progression (TTP), duration of progression free survival (PFS), the response rates (RR), duration of response, time in remission, and/or quality of life. The therapeutically effective amount may improve disease free survival (DFS), improve overall survival (OS), decrease likelihood of recurrence, extend time to recurrence, extend time to distant recurrence (*i.e.*, recurrence outside of the primary site), cure cancer, improve symptoms of cancer (*e.g.*, as gauged using a cancer specific survey), reduce appearance of second primary cancer, *etc.* In some embodiments, the therapeutically effective amounts of one or more drug(s) as disclosed herein are synergistic, *e.g.*, they have a more than additive effect or produce effects that cannot be produced by either agent alone.

[0043] The terms “cancer” and “cancerous” refer to or describe the physiological condition in animal cells that is typically characterized by unregulated cell growth. Included in this definition are malignant cancers as well as dormant tumors or micrometastases. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include lung cancers such as small-cell lung cancer and non-small cell lung cancer, as well as breast, gynecological, and gastrointestinal cancer.

[0044] The term “lung cancer” or “lung cancer cells” in the present methods includes, but is not limited to, the three main types and corresponding subtypes of lung cancer. Examples of types and subtypes of lung cancer include, but are not limited to, non-small cell lung cancer (including squamous cell carcinoma, adenocarcinoma, and large cell carcinoma), small cell lung cancer (including oat cell cancer), and lung carcinoid tumor. In some embodiments, the present methods include treating lung cancer/lung cancer cells including non-small cell adenocarcinoma lung cancer. In other embodiments, the present methods include treating small-cell lung cancer.

[0045] The term “breast cancer” or “breast cancer cells” in the present methods includes, but is not limited to, *in situ* (*e.g.*, ductal carcinoma), invasive (*e.g.*, ductal and lobular carcinoma), and inflammatory breast cancer.

[0046] The term “gynecological cancer” or “gynecological cancer cells” in the present methods includes, but is not limited to, ovarian (*e.g.*, Type I and Type II carcinomas), cervical (*e.g.*, squamous cell carcinoma, adenocarcinoma, adenosquamous carcinoma, and small cell

carcinoma), uterine/endometrial (*e.g.*, Type I and Type II carcinomas including endometrioid carcinoma), vaginal, and vulvar cancer.

[0047] The term “gastrointestinal cancer” or “gastrointestinal cancer cells” in the present methods includes, but is not limited to, esophageal (*e.g.*, adenocarcinoma and squamous cell carcinoma), stomach, liver (*e.g.*, hepatocellular carcinoma and intrahepatic bile duct carcinoma), pancreas (*e.g.*, exocrine and neuroendocrine including adenocarcinoma), small intestine, large intestine, rectum, and anus cancer.

[0048] The term “metastasis” refers to the spread of cancer from its primary site to other places in the body. Cancer cells can metastasize by breaking away from a primary tumor, penetrating into lymphatic and blood vessels, circulating through the lymphatic system or bloodstream, exiting the lymph or blood vessels and attaching and growing in normal tissues elsewhere in the body. Metastasis can be local or distant. At the new site, the cells reproduce, may establish a blood supply and can grow to form a secondary tumor, *i.e.*, a metastatic tumor. Both stimulatory and inhibitory molecular pathways within the tumor cell regulate this behavior, and interactions between the tumor cell and host cells in the distant site are also significant.

[0049] The term “micrometastasis” refers to a small number of cells that have spread from the primary tumor to other parts of the body. Micrometastasis may or may not be detected in a screening or diagnostic test.

[0050] “Molecular weight” as used herein with respect to polymers refers to number average molecular weights (M_n) and can be determined by techniques well known in the art including gel permeation chromatography (GPC).

[0051] The term “tumor” refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

[0052] The term “inhibit” or “inhibition” or “inhibiting,” in the context of neoplasia, tumor growth, or tumor or cancer cell growth. For example, inhibition may include, but is not limited to, delayed appearance of primary or secondary tumors, slowed development of primary or secondary tumors, decreased occurrence of primary or secondary tumors, slowed or decreased severity of secondary effects of disease, arrested tumor growth and regression of tumors, among others. In the extreme, complete inhibition can be referred to as prevention, chemoprevention, tumor regression, or complete tumor regression. The inhibition can be about 10%, about 25%,

about 50%, about 75%, or about 90% inhibition, with respect to progression that would occur in the absence of treatment.

[0053] The term “regression” or “cancer cell regression” refers to the partial or complete disappearance of cancerous cells and tissues.

[0054] The term “subject” or “patient” refers to a mammal, such as a cat, dog, rodent or primate. Typically the subject is a human, and, preferably, a human having or suspected of having a cancer such as lung cancer. The term “subject” and “patient” can be used interchangeably.

[0055] As will be understood by one skilled in the art, for any and all purposes, particularly in terms of providing a written description, all ranges disclosed herein also encompass any and all possible subranges and combinations of subranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, *etc.* As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, *etc.* As will also be understood by one skilled in the art, all language such as “up to,” “at least,” “greater than,” “less than,” and the like include the number recited and refer to ranges which can be subsequently broken down into subranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, an amount of a compound of 1-3 mg refers to 1, 2, or 3 mg of the compound. Similarly, an amount of a compound of 1-5 mg refers to 1, 2, 3, 4, or 5 mg of the compound, and so forth.

[0056] The term “micelle” refers to a particle of colloidal dimensions that exists in equilibrium with the molecules or ions in solution from which it is formed. Generally, examples of micelles include, but are not limited to, lipid-like molecules that aggregate in aqueous solution to a spherical form. Micelle formation in solution occurs by a process called micellization, in which surface-active molecules or ions aggregate to form micelles. For example, micelles can be formed from amphiphilic molecules or polymers. The present methods and compositions include micelles, a core-shell structure formed from amphiphilic polymers that contains a lipophilic core that is capable of encapsulation. For example, poly(ethylene glycol)-block-poly(lactic acid) are a type of amphiphilic-block-copolymer that forms micelles.

[0057] The terms "PEG-PLA" or "PEG-b-PLA" refers to poly(ethylene glycol)-block-poly(lactic acid). The poly(ethylene glycol) (PEG) or poly(ethylene oxide) block is a polymer (or oligomer) of ethylene oxide prepared by polymerization of ethylene oxide. PEG is commercially available at molecular weights of about 300 g/mol to about 10,000,000 g/mol. In the present technology, PEG is used as the hydrophilic block of amphiphilic copolymers to create micelles, and other types of artificial vesicles. The poly(lactic acid) (PLA) block can include (D-lactic acid), (L-lactic acid), (D,L-lactic acid), or any combination thereof. PLA is a polyester of lactic acid having various well-known synthetic preparations. PLA often serves as the hydrophobic block of amphiphilic copolymers to create micelles, and other types of artificial vesicles. Various forms of PEG and PLA are available commercially, or they can be prepared according to methods well-known to those of skill in the art.

[0058] The term "drug loading" refers to the capacity for encapsulating the drug combinations of paclitaxel, 17-AAG, and rapamycin, by micelle. Drug loading for multidrug micelles can approach, be equal to, or exceed the drug loading capacity of single agent micelles.

[0059] The terms "chemotherapeutic agent," "chemotherapy agent," "chemotherapy drug," or "chemotherapeutic drug" refer to an agent that reduces, prevents, mitigates, limits, and/or delays the growth of metastases or neoplasms, or kills neoplastic cells directly by necrosis or apoptosis of neoplasms or any other mechanism, or that can be otherwise used, in a pharmaceutically-effective amount, to reduce, prevent, mitigate, limit, and/or delay the growth of metastases or neoplasms in a subject with neoplastic disease. Chemotherapeutic agents include chemical compounds useful in the treatment of cancer. Examples of chemotherapeutic agents, but are not limited to, Taxol and paclitaxel.

[0060] The examples herein are provided to illustrate advantages of the present technology and to further assist a person of ordinary skill in the art with using the present technology. The examples should in no way be construed as limiting the scope of the present technology, as defined by the appended claims. The examples can include or incorporate any of the variations, aspects or aspects of the present technology as described herein. The variations, aspects or aspects described herein may also further include or incorporate variations of any or all other variations, aspects or aspects of the present technology.

EXAMPLES

[0061] Abbreviations.

CI	Combination index
Hsp90	Heat shock protein 90
IV	Intravenous
mTOR	mammalian target of rapamycin
NSCLC	Non-small-cell lung cancer
PDI	Polydispersity index
PEG- <i>b</i> -PLA	Poly(ethylene glycol)- <i>block</i> -poly(D,L-lactic acid)
PTX or P	Paclitaxel
PTX-CBT	Paclitaxel-containing PEG- <i>b</i> -PLA micelle combined with carboplatin
RAPA or R	Rapamycin
17-AAG or 17	17-Allylamino-17-demethoxy-geldanamycin
CBT or C	Carboplatin
TRIO	Triolimus
TRIO-CBT	Triolimus combined with carboplatin

Materials.

[0062] A549 human NSCLC cell line was purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). A549 cells were cultured in RPMI medium supplemented with 10% heat inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were maintained at 37°C in a humidified 5% CO₂ atmosphere. Phosphate-buffered saline (PBS), penicillin/ streptomycin, trypsin-EDTA, RPMI medium, and fetal bovine serum (FBS) were purchased from Corning (Corning, NY, USA). Acetonitrile and methanol were obtained from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Cell Titer-Blue® reagent was purchased from Promega (Madison, WI, USA). CBT and Propidium iodide (PI) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). PEG-*b*-PLA with M_n of PEG = 4000 g mol⁻¹ and PLA = 2200 g mol⁻¹ was purchased from Advanced Polymer Materials Inc. (Montreal, CAN). PTX, 17-AAG, and RAPA were purchased from LC Laboratories. (Woburn, MA, USA). All chemicals were analytical grade.

General Procedures.

1: Preparation and characterization of TRIO PEG-*b*-PLA micelles ("TRIO") and TRIO PEG-*b*-PLA micelles and CBT (TRIO-CBT)

[0063] 52.5 mg of PEG-*b*-PLA and 3.0 mg of PTX, 3.0 mg of 17-AAG, and 1.5 mg of RAPA were dissolved in 1.0 mL of *tert*-butanol at 60 °C, followed by addition of 1.0 mL of pre-warmed double-distilled water at 60 °C with vigorous mixing and frozen in dry ice/ethanol

cooling bath at -70 °C for 2 h. Lyophilization was performed on a VirTis AdVantage Pro freeze dryer (SP Scientific, Gardiner, NY, USA) at -20 °C shelf inlet temperature for 72 h at 100 μ Bar. The freeze-dried samples were stored at -20 °C until use. Before dosing, lyophilized cake of TRIO was rehydrated with 1.0 mL of 0.9% saline solution at 60 °C, centrifuged at 13,000 rpm for 5 min, and filtered through 0.22 μ m regenerated cellulose filter. In TRIO, mass or molar ratio of PTX: 17-AAG: RAPA was 2:2:1 or 2:3:1, respectively, enabling mouse dosing of PTX, 17-AAG, and rapamycin at 15, 15, 7.5 mg/kg, respectively. CBT was added to reconstituted TRIO, forming TRIO-CBT for injection.

[0064] Z-average diameter and polydispersity index (PDI) of TRIO and TRIO-CBT at 25 °C were measured using a Zetasizer Nano-ZS (Malvern Instruments, UK) at a fixed angle of 173°. Prior to the measurement, TRIO and TRIO-CBT were diluted 50-fold with physiological saline.

[0065] The drug content of PEG-*b*-PLA micelles was quantified by a reverse-phase Shimadzu Prominence HPLC system (Shimadzu, Japan). Ten μ L of lyophilized sample was dissolved in 990 μ L of acetonitrile. Ten μ L of the dissolved solution was injected into a Zorbax RX-C8 analytical column (4.6 mm $\times$ 250 mm, particle size 5 μ m, Agilent) at a flow rate of 1.0 mL/min, a run time of 20 minutes, and a column oven temperature of 40 °C. The separation of PTX, 17-AAG, and RAPA was achieved with a mobile phase consisting of 55% of acetonitrile, and 45% of water containing 0.1% phosphoric acid and 1% methanol under isocratic conditions. PTX, 17-AAG, and RAPA were detected at 227, 333, and 279 nm, respectively. Retention times of PTX, 17-AAG, and RAPA were 3, 4, and 11 minutes, respectively.

2: A549 cell culture

[0066] A549 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. A549 cells were maintained at 37 °C under an atmosphere of 5% CO₂ in a humidified incubator.

3: *In vitro* cytotoxicity assay

[0067] Cell viability was measured using Cell Titer-Blue® viability assay. Briefly, A549 cells were seeded onto 96-well plates and incubated overnight at 37 °C in 5% CO₂. The next day, the medium in each well was replaced with fresh medium containing saline control, single drug-loaded PEG-*b*-PLA micelle (PTX, 17-AAG, RAPA), 2- and 3-drug loaded-PEG-*b*-PLA micelles (PTX-17-AAG, PTX-RAPA, 17-AAG-RAPA, PTX-17-AAG-RAPA with molar ratio 1:1, 1:1,

1:1, 2:3:1) or each plus CBT (*see* Table 3 for the drug molar ratio combinations) at 0 to 10,000 nM. After 72 hrs incubation, culture medium was removed, and serum-free medium containing 20% Cell Titer-Blue® reagent was added to each well. The cells were incubated for 1 h followed by measuring the fluorescence intensity of the solution in each well with a Spectra M2 plate reader (Molecular Device, Sunnyvale, CA, USA) with an excitation wavelength (λ_{ex}) of 560 nm and an emission wavelength (λ_{em}) of 590 nm. The drug concentration that induced a 50% inhibition of cell growth (IC_{50}) was calculated by curve fitting.

4: Combination index analysis

[0068] Combination index (CI) values were calculated using CompuSyn software according to Chou's method to assess drug interaction (*see* T.C. Chou, *et al.*, "Quantitation of the synergistic interaction of edatrexate and cisplatin *in vitro*," Cancer Chemother Pharmacol. 1993; 13:259-264). CI values were obtained from:

$$\text{Combination index (CI)} = \left[\frac{A}{A_x} \right] + \left[\frac{B}{B_x} \right] + \left[\frac{C}{C_x} \right]$$

where A, B, and C are concentrations of drug A, B, and C in a combination that inhibits cell growth by x% (isoeffective compared with the individual drugs), and A_x , B_x , and C_x are the concentrations of drug A, B, and C, which induce x% inhibition of cell growth. Values of $CI > 1$, $CI = 1$, and $CI < 1$ indicate antagonism, additive, and synergism, respectively.

5: Cell cycle analysis

[0069] A549 cells were seeded overnight in a 6-well plate with RPMI medium containing 10% FBS (3×10^5 cells/well). The next day, the cells were treated with control, drug-loaded-PEG-*b*-PLA micelles (PTX, 17-AAG, RAPA, TRIO) at 100 nM or PTX-loaded-PEG-*b*-PLA micelles plus CBT or TRIO plus CBT (PTX-CBT, TRIO-CBT with molar ratio 2:5, 2:3:1:5, respectively) at 100 nM. After 24 hrs, cells were harvested by trypsinization and centrifugation at $1,000 \times g$ for 5 min, cell pellets were washed with cold PBS and fixed gently by 70% ethanol at $-20^\circ C$ overnight. The cells were then washed with cold PBS and resuspended in PBS containing 20 $\mu g/mL$ PI and 0.1 mg/mL RNase A in the dark for 30 min at room temperature until the cells were analyzed by flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA, USA) equipped with a 488 nm laser. Cell cycle distribution and sub- G_1 (apoptosis) group were determined and analyzed by FlowJo software (Tree Star Inc., Ashland, OR, USA).

6: Acute toxicity

[0070] Toxicity was performed on healthy BALB/c mice (6-8 week-old), purchased from Harlan Laboratories (Madison, WI, USA), following a schedule of daily IV injections of PTX-loaded micelle, TRIO or TRIO-CBT for three days. All animal experiments were approved by the Institutional Animal Care and Use Committee and conducted in accordance with institutional and NIH guidance. BALB/c mice were divided into 4 groups: (i) Control (saline); (ii) PTX-loaded PEG-*b*-PLA micelles (50 mg/kg, maximal tolerated dose (MTD)) (iii) TRIO with PTX, 17-AAG, and RAPA at 60, 60 and 30 mg/kg, respectively; and (iv) TRIO-CBT with PTX, 17-AAG, RAPA, and CBT at 60, 60, 30 and 60 mg/kg, respectively. After three-daily dose schedule (tail-vein injections on day 0, 1, 2), the body weight changes of mice were regularly monitored for six days and evaluated in comparison with the control group.

7: Antitumor efficacy

[0071] A549 NSCLC tumor xenograft model was established in athymic female nude mice (6-8 week-old), purchased from Harlan Laboratories (Madison, WI, USA). All animal experiments were approved by the Institutional Animal Care and Use Committee and conducted in accordance with institutional and NIH guidance. A549 cells (2×10^6 cells/mouse) were inoculated subcutaneously in the right flank. For tumor growth delay studies, drug treatment was started when tumor volume reached approximately 200 mm³ for A549 tumors. A549 tumor-bearing mice were divided into 4 groups: (i) Control (saline); (ii) PTX-loaded PEG-*b*-PLA micelles (15 mg/kg) plus CBT at 15 mg/kg (PTX-CBT); (iii) TRIO with PTX, 17-AAG, and RAPA at 15, 15 and 7.5 mg/kg, respectively; and (iv) TRIO-CBT with PTX, 17-AAG, RAPA, and CBT at 15, 15, 7.5, and 15 mg/kg, respectively. Dose schedule: 3-weekly tail vein injections and 1 week off for 3-cycles. Tumor volume was monitored by caliper measurement and calculated by: Tumor volume = $0.5 \times (\text{large diameter}) \times (\text{small diameter})^2$. Body weight and survival were recorded and evaluated over the course of this study.

8: Statistical analysis

[0072] Data are presented as mean $\pm$ SE. Multi-group comparisons were conducted by ANOVA. When appropriate, Tukey's honestly significant difference (HSD) test was applied to account for multiple comparisons. For two group comparisons, student t-test was used, and differences were regarded significant for *P*-values < 0.05. Kaplan–Meier survival curve was

generated and the difference in survival time between 2 groups was analyzed with the log-rank test.

Example 1: Physiochemical characterization of TRIO and TRIO-CBT

[0073] CBT did not affect the particle size, polydispersity index (PDI) and loading efficiency of TRIO at room temperature over 24 hrs (Table 1), indicating compatibility and stability of TRIO-CBT for IV infusion. TRIO had a hydrodynamic diameter of 30.3 ± 0.5 nm, low polydispersity (< 0.2), and high drug loading efficiency. The physiochemical properties of TRIO were consistent with a previous report (*see* K. Tomoda, *et al.*, "Triolimus: A multi-drug loaded polymeric micelle containing Paclitaxel, 17-AAG, and Rapamycin as a novel radiosensitizer," *Macromol. Biosci.* 2017, 17, 1600194). In addition, CBT did not affect the particle size, polydispersity index (PDI) and loading efficiency of single-drug or multi-drug-loaded PEG-*b*-PLA micelles (Table 1). For TRIO-CBT for injection, PTX, 17-AAG, and RAPA were at 3.0, 3.0, and 1.5 mg/mL, respectively, and CBT was at 3.0 mg/mL, enabling mouse dosing of PTX, 17-AAG, and RAPA at 15, 15, 7.5 mg/kg, respectively.

Table 1. Summary of physicochemical properties of TRIO and TRIO-CBT.

Micelle type	Initial concentration (mg/mL)	w/o CBT			w/CBT		
		Micelle size (nm)	PDI	Drug loading efficiency (%)	Micelle size (nm)	PDI	Drug loading efficiency (%)
PTX	3	34.3 ± 0.5	0.2 ± 0.02	95.1 ± 0.5	40.9 ± 4.5	0.2 ± 0.02	93.6 ± 0.02
17-AAG	3	242.3 ± 15.9	0.5 ± 0.01	90.6 ± 0.3	231.8 ± 51.6	0.5 ± 0.03	89.3 ± 0.2
RAPA	1.5	50.4 ± 0.8	0.5 ± 0.03	89.6 ± 0.0	59.4 ± 15.4	0.3 ± 0.1	90.0 ± 0.4
PTX:17-AAG	3:3	29.8 ± 4.4	0.3 ± 0.2	97.8 ± 0.6	30.9 ± 0.03	0.1 ± 0.04	92.8 ± 0.2
PTX:RAPA	3:1.5	36.7 ± 0.2	0.3 ± 0.02	97.8 ± 0.5	39.1 ± 1.1	0.3 ± 0.04	94.1 ± 0.6
17-AAG:RAPA	3:1.5	87.6 ± 21.3	0.4 ± 0.2	97.0 ± 0.5	94.6 ± 37.3	0.4 ± 0.2	94.8 ± 0.5
PTX:17-AAG:RAPA	3:3:1.5	30.3 ± 0.5	0.2 ± 0.01	93.4 ± 0.4	30.2 ± 3.0	0.3 ± 0.1	96.0 ± 0.6

Example 2: *In vitro* cytotoxicity of TRIO and TRIO-CBT

[0074] Single-drug, 2-drug loaded-PEG-*b*-PLA micelles, and TRIO displayed varied cytotoxicity against human NSCLC A549 cancer cells with IC₅₀ values, ranging from 5 nM to 20 μ M (Table 2). After 72 hours, 2- and 3-drug combinations exerted higher cytotoxicity compared to single-drug loaded-PEG-*b*-PLA micelles. TRIO had the lowest IC₅₀ value of 5.1 ± 1.5 nM (indicating the most cytotoxic against A549 cells). Consistent with earlier results, CI values of 2-drug loaded-PEG-*b*-PLA micelles and TRIO were less than 1 ($CI < 1$) from 25 to 90 % fraction affected (Fa).

Table 2. *In vitro* cytotoxicity of TRIO versus single drug and 2-drug combinations for A549 cells.

Micelle type	IC ₅₀ (nM)	CI at F ₂₅	CI at F ₅₀	CI at F ₇₅	CI at F ₉₀	Molar ratio
PTX	97.4 ± 4.5	-	-	-	-	
17-AAAG	16.9 ± 0.8	-	-	-	-	
RAPA	20460 ± 0.7	-	-	-	-	
PTX:17-AAAG	14.5 ± 0.9	0.02 ± 0.01	0.1 ± 0.01	0.04 ± 0.02	0.02 ± 0.3	1:1
PTX:RAPA	1936 ± 2.7	0.1 ± 0.02	0.01 ± 0.02	0.1 ± 0.01	0.1 ± 0.02	1:1
17-AAAG:RAPA	17.8 ± 1.4	0.1 ± 0.1	0.1 ± 0.1	0.01 ± 0.1	0.1 ± 0.1	1:1
PTX:17-AAAG:RAPA	5.1 ± 1.5	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.4	2:3:1

[0075] TRIO-CBT (Table 3) exhibited higher or similar cytotoxicity against A549 cells in comparison with TRIO (Table 2), even though the IC₅₀ value of CBT was high (about 4 μ M). PTX-CBT had an IC₅₀ value at 13 ± 8.8 nM. CI values of CBT combined with single-drug-, 2- or 3-drug-loaded-PEG-*b*-PLA micelles indicated synergistic cytotoxicity effects against A549 cells for most Fa. Importantly, TRIO-CBT had the lowest IC₅₀ value at 2.8 ± 1.6 nM, indicating potency for PTX and CBT, combined with mTOR/Hsp90 inhibition (Table 3).

Table 3. *In vitro* cytotoxicity of TRIO-CBT versus single drug, 2-drug, and TRIO for A549 cells.

Micelle type	IC ₅₀ (nM)	CI at F ₂₅	CI at F ₅₀	CI at F ₇₅	CI at F ₉₀	Molar ratio
CBT	3708 ± 3.3					
PTX-CBT	13 ± 8.8	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.02	0.02 ± 0.03	2:5
17-AAG-CBT	12.9 ± 0.4	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	3:5
RAPA-CBT	3247 ± 2.0	7.2 ± 3.6	1.6 ± 0.2	0.5 ± 0.5	0.2 ± 0.3	1:5
PTX:17-AAG:CBT	15.6 ± 1.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.04 ± 0.03	2:3:5
PTX:RAPA:CBT	7.1 ± 3.3	0.01 ± 0.02	0.01 ± 0.03	0.02 ± 0.01	0.02 ± 0.02	2:1:5
17-AAG:RAPA:CBT	17.2 ± 0.9	0.1 ± 0.1	0.1 ± 0.1	0.04 ± 0.1	0.03 ± 0.02	3:1:5
PTX:17-AAG:RAPA:CBT	2.8 ± 1.6	0.1 ± 0.04	0.1 ± 0.03	0.1 ± 0.03	0.04 ± 0.03	2:3:1:5

Example 3: Cell cycle analysis of TRIO and TRIO-CBT

[0076] To explore mechanisms underlying the potent cytotoxicity of TRIO-CBT on A549 cells, cell cycle analysis was performed by flow cytometry and biochemical analysis was performed by Western blot. Comparisons were made with TRIO and PTX-CBT, as first-line chemotherapy for NSCLC. While not wishing to be bound by theory, the data suggests that TRIO-CBT induces cell cycle arrest at G2/M phase and apoptosis by simultaneously inhibiting MAPK and PI3K/Akt/mTOR signaling pathways.

Example 4: Acute toxicity of TRIO and TRIO-CBT

[0077] The toxicity assay was conducted on BALB/c healthy mice (6-8 week-old) using 3 daily IV injections followed by body weight measurement for six days. As shown in FIG. 1, overall body weight loss was in the following order: PTX-loaded micelle (50 mg/kg) > TRIO-CBT (PTX:17-AAG:RAPA:CBT, 60:60:30:60 mg/kg) > TRIO (PTX:17-AAG:RAPA, 60:60:30 mg/kg) > Control. After the completion of treatment, the body weight value (80%) of PTX-loaded micelle group was significantly lower ($P < 0.05$) than TRIO, TRIO-CBT and the untreated control groups, defining the MTD of the PTX-loaded micelle at 50 mg/kg injected daily for 3 days (FIG. 1). TRIO and TRIO-CBT had 2-10% body weight loss, indicating that TRIO and TRIO-CBT are comparably safer, perhaps owing to higher physical stability of TRIO over PTX-loaded micelle *in vivo* (see H. Cho, *et al.* "PEG-*b*-PLA micelles and PLGA-*b*-PEG-*b*-PLGA sol-gels for drug delivery," J Control Release. 2016; 240:191-201).

Example 5: Antitumor activity of TRIO and TRIO-CBT

[0078] TRIO and TRIO-CBT induced tumor regression in an A549 tumor xenograft model, whereas PTX-CBT largely inhibited tumor growth (FIGS. 2A-2C). After termination of treatment, there were tumor recurrences after 15 weeks for PTX-CBT and TRIO. However, there was no relapse for Trio-CBT (FIG. 2A). TRIO-CBT prolonged the survival of mice as compared with control and PTX-CBT groups (FIG. 2B). The high antitumor efficacy of TRIO in an A549 xenograft model is consistent with earlier results in breast, NSCLC, and ovarian tumor xenograft models (see H. Cho, *et al.*). The higher antitumor activity of TRIO-CBT over PTX-CBT is consistent with *in vitro* results on proliferation and apoptosis and point to the value of mTOR/Hsp90 inhibition for increasing the potency of the doublet of PTX and CBT. As shown in FIG. 2C, there was no significant body weight change in mice after TRIO-CBT

treatment, indicating that mTOR/Hsp90 inhibition does not markedly increase the toxicity of PTX and CBT.

[0079] Examples 1-5 demonstrate TRIO-CBT as a 4-drug combination can be easily and safely attained as combined TRIO with CBT in sterile water for injection. TRIO-CBT was stable in sterile water for injection *in vitro* and induced synergistic anticancer activity *in vitro* and potent antitumor efficacy *in vivo*.

WHAT IS CLAIMED IS:

1. A method of treatment comprising administering to cancer cells a therapeutically effective amount of carboplatin in conjunction with a micelle composition comprising a therapeutically effective amount of paclitaxel, 17-AAG, and rapamycin;
wherein:
the cancer cells comprise lung, breast, gynecological, or gastrointestinal cancer cells, or a combination of two or more thereof;
the weight ratio of paclitaxel, 17-AAG, rapamycin, and carboplatin is about 1:1:1:1 to about 5:5:1:10; and
the micelle composition comprises poly(ethylene glycol)-block-poly(lactic acid) polymers.
2. The method of claim 1, wherein the method of treatment comprises administering a composition comprising the carboplatin and the micelle composition.
3. The method of claim 1 or claim 2, wherein the weight ratio of paclitaxel, 17-AAG, rapamycin, and carboplatin is about 1:1:1:1 to about 5:5:1:8.
4. The method of any one of claims 1-3, wherein the weight ratio of paclitaxel, 17-AAG, rapamycin, and carboplatin is about 2:2:1:2 to about 2:3:1:5.
5. The method of any one of claims 1-4, wherein the therapeutically effective amount of each of paclitaxel and 17-AAG is about 2 mg/kg to about 90 mg/kg and the therapeutically effective amount of rapamycin is about 1 mg/kg to about 50 mg/kg.
6. The method of any one of claims 1-5, wherein the therapeutically effective amount of each of paclitaxel and 17-AAG is about 40 mg/kg to about 75 mg/kg and the therapeutically effective amount of rapamycin is about 20 mg/kg to about 40 mg/kg.
7. The method of any one of claims 1-6, wherein the therapeutically effective amount of carboplatin is about 2 mg/kg to about 90 mg/kg.
8. The method of any one of claims 1-7, wherein the therapeutically effective amount of carboplatin is about 40 mg/kg to about 75 mg/kg.

9. The method of any one of claims 1-5, wherein the therapeutically effective amount of each of paclitaxel and 17-AAG is about 10 mg/kg to about 25 mg/kg and the therapeutically effective amount of rapamycin is about 3 mg/kg to about 15 mg/kg.
10. The method of claim 9, wherein the therapeutically effective amount of carboplatin is about 10 mg/kg to about 25 mg/kg.
11. The method of any one of claims 1-10, wherein the cancer cells comprise lung cancer cells.
12. The method of claim 11, wherein the lung cancer cells comprise non-small cell lung cancer cells.
13. The method of any one of claims 1-10, wherein the cancer cells comprise breast, gynecological, or gastrointestinal cancer cells.
14. The method of any one of claims 1-13, wherein the combined drug loading of paclitaxel, 17-AAG, and rapamycin in the micelles is about 5 wt. % to about 40 wt.%.
15. The method of any one of claims 1-14, wherein the individual drug loading of paclitaxel, 17-AAG, and rapamycin in the micelles is about 1 wt. % to about 25 wt.%.
16. The method of any one of claims 1-15, wherein the PEG-b-PLA comprises a poly(ethylene glycol)-block having a molecular weight of about 1,500 to about 30,000 g/mol and a poly(lactic acid) block having a molecular weight of about 1,500 to about 30,000 g/mol.
17. A method of treatment comprising administering to a subject having lung, breast, gynecological, gastrointestinal cancer, or a combination of two or more thereof, a therapeutically effective amount of carboplatin and a therapeutically effective amount of a micelle composition comprising paclitaxel, 17-AAG, and rapamycin;
wherein:
the weight ratio of paclitaxel, 17-AAG, rapamycin, and carboplatin is about 1:1:1:1 to about 5:5:1:10; and
the micelle composition comprises poly(ethylene glycol)-block-poly(lactic acid) polymers.
18. The method of claim 17, wherein the subject is a mammal.

19. A composition comprising carboplatin and a micelle composition comprising paclitaxel, 17-AAG, and rapamycin;

wherein:

the weight ratio of paclitaxel, 17-AAG, rapamycin, and carboplatin is about 1:1:1:1 to about 5:5:1:10; and

the micelle composition comprises poly(ethylene glycol)-block-poly(lactic acid) polymers.

20. A kit comprising carboplatin and a micelle composition comprising paclitaxel, 17-AAG, and rapamycin;

wherein:

the weight ratio of paclitaxel, 17-AAG, rapamycin, and carboplatin is about 1:1:1:1 to about 5:5:1:10; and

the micelle composition comprises poly(ethylene glycol)-block-poly(lactic acid) polymers.

FIG. 1

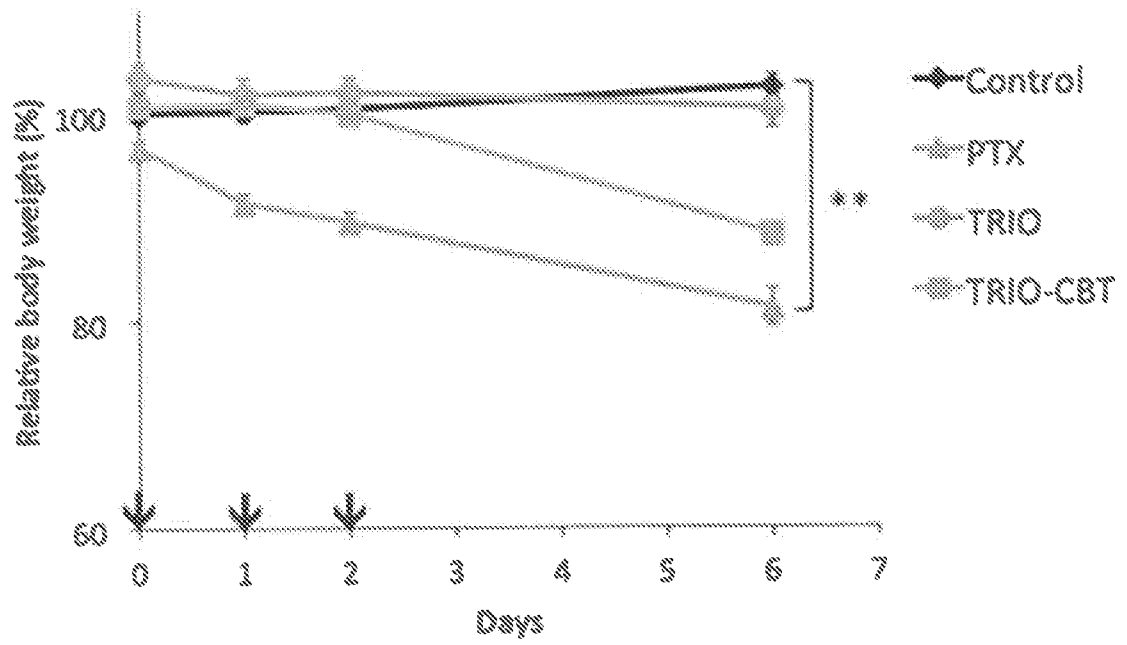


FIG. 2A

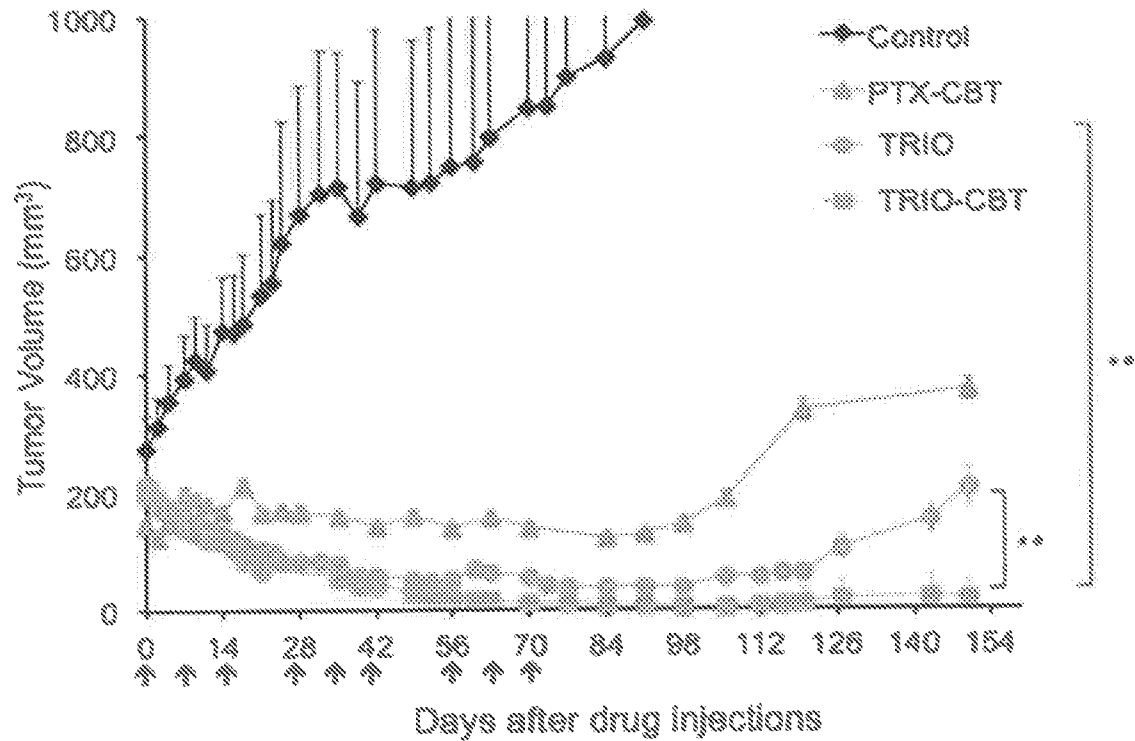


FIG. 2B

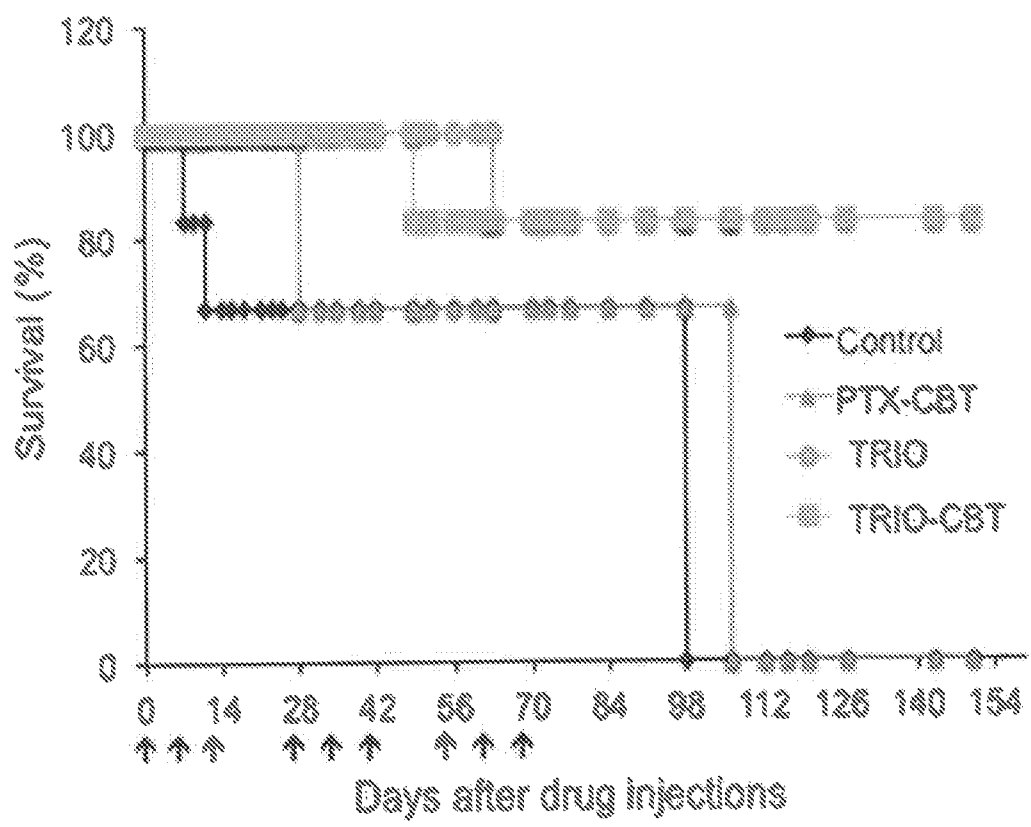
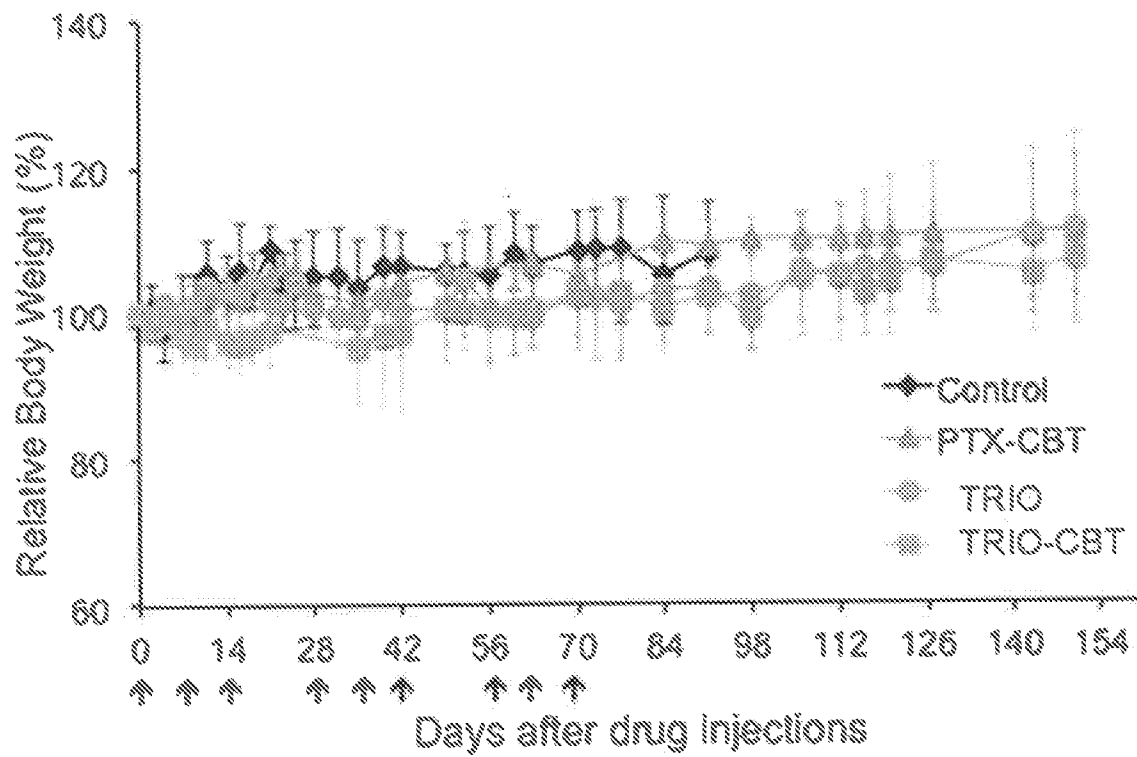


FIG. 2C



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/049574

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/107 A61K31/282
ADD.

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)
A61K

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

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

EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. R. HASENSTEIN ET AL: "Antitumor Activity of Triolimus: A Novel Multidrug-Loaded Micelle Containing Paclitaxel, Rapamycin, and 17-AAG", MOLECULAR CANCER THERAPEUTICS, vol. 11, no. 10, 2012, pages 2233-2242, XP055289416, page 2234, left-hand column, paragraph 1; figure 1	1-20
A	CHO HYUNAH ET AL: "Polymeric Micelles for Multi-Drug Delivery in Cancer", AAPS PHARMSCITECH, vol. 16, no. 1, 2014, pages 10-20, XP035437685, page 10, right-hand column, paragraph 3; figure 6 ----- -/--	1-20



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"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

11 December 2019

Date of mailing of the international search report

18/12/2019

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/049574

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HO-CHUL SHIN ET AL: "A 3-in-1 Polymeric Micelle Nanocontainer for Poorly Water-Soluble Drugs", MOLECULAR PHARMACEUTICS, vol. 8, no. 4, 2011, pages 1257-1265, XP055649865, the whole document	1-20
A	----- WO 2014/168845 A1 (IGDRASOL [US]) 16 October 2014 (2014-10-16) paragraph [0079] -----	1-20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2019/049574

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014168845 A1	16-10-2014	US 2015366806 A1 WO 2014168845 A1	24-12-2015 16-10-2014
