PENTAMIDINE COMBINATIONS FOR TREATING CANCER

The present invention relates to synergistic combinations of chemotherapeutic agents for treating cancer.

There is a need for agents and combinations thereof that inhibit the proliferation of cancer cells that are less toxic and/or more active than conventional chemotherapeutics, e.g., especially where an agent or combination of agents permits the use of lower dosages of chemotherapeutics administered to cancer patients without loss of therapeutic efficacy.

SUMMARY OF THE INVENTION

The present invention is related to U.S. Patent No. 7,115,665, which discloses use of pentamidine to treat cancer. It is incorporated herein in its entirety.

One aspect of the present invention is a method of inhibiting the proliferation of cancer cells comprising administering to a patient in need thereof (1) pentamidine and (2) (a) oxaliplatin, (b) gemcitabine, (c) taxol, (d) 5-fluorouracil or (e) CPT 11 (camptothecin-11, also known as Irinotecan). The agents can be given either separately, for example on consecutive days, or together.

According to another aspect of the present invention, the method inhibits the proliferation of cancer cells and tumour growth.

According to another aspect of the present invention, there is provided a pharmaceutical composition for inhibiting the proliferation of cancer cells and/or tumour growth that comprises a combination of the compounds above. The invention relates to the surprising discovery that the combinations are synergistic.

In a preferred aspect, the cancer cells are squamous cell carcinoma cells, larger cell carcinoma of the lymph node cells, breast cancer cells, colon cancer cells, lung carcinoma cells, melanoma cells, pancreatic cancer cells, leukemia cells, non-small cell lung cancer cells, colon cancer cells, central nervous system (CNS) cancer cells, ovarian cancer cells, renal cancer cells or prostate cancer cells.

In a preferred aspect, the cancer cells are pancreatic cancer cells, colon cancer cells, breast cancer cells or ovarian cancer cells.

In another preferred aspect, pentamidine is combined with gemcitabine, for instance, for treating pancreatic cancer, or is used alone for such purpose; or pentamidine is combined with oxaliplatin, for instance, for treating colon cancer. For the treatment of (advanced or metastatic) breast or ovarian cancer, doxorubicin, 5-fluorouracil, carboplatin, and paclitaxel are examples of components of standard chemotherapy regimens. Capecitabine (Xeloda®), an orally administered systemic pro-drug of 5'-deoxy-5-fluorouridine (5'DFUR) which is converted to 5-fluorouracil, is also used. While these treatments have extended survival, patients eventually experience disease progression. The incorporation of pentamidine in combination with standard chemotherapy, for example, doxorubincin or 5-fluorouracil or carboplain or paclitaxel, comprises another aspect of this invention.

Pentamidine refers to the free compound or to the compound in salt form, e.g., as the commercially available pentamidine isethionate, or any other pharmaceutically acceptable salt.

The present invention also relates to the further combination of the above agent combinations with additional agents that cause DNA breaks. Including these types of agents provides a valuable tool for cancer therapy. Agents that induce DNA breaks that are within the scope of the present invention include but are not limited to cisplatin, mitomycin C, melphalan, carmustine, adriamycin, taxol, 5-fluorouracil, bevacizumab, capecitabine, folinic

acid (also known as leucovorin), ionizing irradiation and bleomycin or with any agent 2(a), 2(b) or 2(c) not in the above combination. Without wishing to be bound by theory, such combinations are believed to operate in view of the inhibition of endo-exonuclease activity by pentamidine. (Other endo-exonuclease activity inhibitors can also be used together with or in place of pentamidine, such as distamycin A and berenil). Such inhibition prevents repair of double-breaks induced directly or indirectly by the mentioned DNA break-inducing agents. The mentioned DNA break-inducing agents can cause double strand breaks directly or can cause single strand breaks that progress to double strand breaks. This is a common occurrence in biological systems. The endo-exonuclease inhibitors such as pentamidine prevent double break repair and thus enhance anticancer effects.

Compositions or mixtures of the disclosed compound combinations may be administered to patients, which include humans and animals. Such compositions or formulations are conventionally prepared. Compositions include all pharmaceutical formulations of a compound and a compound in its pure state. Combinations can include two or more compositions of the individual agents. These include two or more different formulations of a compound such as a tablet formulation for one agent and a liquid formulation for another. Mixtures of two or more compounds in the same formulation are also within the scope of the invention. Compositions also include the usual conventional adjuvants/excipients well known in the pharmaceutical field.

Pharmaceutical formulations can thus be adapted for administration via any desired suitable method, preferably by fully conventional methods, for example by oral (including buccal or sublingual), rectal, nasal, topical (including buccal, sublingual or transdermal), vaginal or parenteral (including subcutaneous, intramuscular, intravenous or intradermal) methods. Such formulations can be prepared using all processes known in the pharmaceutical art by, for example, combining the active ingredient with the excipient(s) or adjuvant(s).

Pharmaceutical formulations adapted for oral administration can be administered as separate units, such as, for example, capsules or tablets; powders or granules; solutions or suspensions in aqueous or non-aqueous liquids; edible foams or foam foods; or oil-in-water liquid emulsions or water-in-oil liquid emulsions.

Thus, for example, in the case of oral administration in the form of a tablet or capsule, the active-ingredient component can be combined with an oral, non-toxic and pharmaceutically acceptable inert excipient, such as, for example, ethanol, glycerol, water and the like. Powders are prepared by comminuting the compound to a suitable fine size and mixing it with a pharmaceutical excipient comminuted in a similar manner, such as, for example, an edible carbohydrate, such as, for example, starch or mannitol. A flavour, preservative, dispersant and dye may likewise be present.

Capsules are produced by preparing a powder mixture as described above and filling shaped gelatine shells therewith. Glidants and lubricants, such as, for example, highly disperse silicic acid, talc, magnesium stearate, calcium stearate or polyethylene glycol in solid form, can be added to the powder mixture before the filling operation. A disintegrant or solubiliser, such as, for example, agar-agar, calcium carbonate or sodium carbonate, may likewise be added in order to improve the availability of the medicament after the capsule has been taken.

In addition, if desired or necessary, suitable binders, lubricants and disintegrants as well as dyes can likewise be incorporated into the mixture. Suitable binders include starch, gelatine, natural sugars, such as, for example, glucose or beta-lactose, sweeteners made from maize, natural and synthetic rubber, such as, for example, acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes, and the like. The lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. The disintegrants include,

without being restricted thereto, starch, methylcellulose, agar, bentonite, xanthan gum and the like. The tablets are formulated by, for example, preparing a powder mixture, granulating or dry-pressing the mixture, adding a lubricant and a disintegrant and pressing the entire mixture to give tablets. A powder mixture is prepared by mixing the compound comminuted in a suitable manner with a diluent or a base, as described above, and optionally with a binder, such as, for example, carboxymethylcellulose, an alginate, gelatine or polyvinylpyrrolidone, a dissolution retardant, such as, for example, paraffin, an absorption accelerator, such as, for example, a quaternary salt, and/or an absorbent, such as, for example, bentonite, kaolin or dicalcium phosphate. The powder mixture can be granulated by wetting it with a binder, such as, for example, syrup, starch paste, acadia mucilage or solutions of cellulose or polymer materials and pressing it through a sieve. As an alternative to granulation, the powder mixture can be run through a tableting machine, giving lumps of non-uniform shape which are broken up to form granules. The granules can be lubricated by addition of stearic acid, a stearate salt, talc or mineral oil in order to prevent sticking to the tablet casting moulds. The lubricated mixture is then pressed to give tablets. The compounds according to the invention can also be combined with a free-flowing inert excipient and then pressed directly to give tablets without carrying out the granulation or dry-pressing steps. A transparent or opaque protective layer consisting of a shellac sealing layer, a layer of sugar or polymer material and a gloss layer of wax may be present. Dyes can be added to these coatings in order to be able to differentiate between different dosage units.

Oral liquids, such as, for example, solution, syrups and elixirs, can be prepared in the form of dosage units so that a given quantity comprises a pre-specified amount of the compound. Syrups can be prepared by dissolving the compound in an aqueous solution with a suitable flavour, while elixirs are prepared using a non-toxic alcoholic vehicle. Suspensions can be formulated by dispersion of the compound in a non-toxic vehicle. Solubilisers and

emulsifiers, such as, for example, ethoxylated isostearyl alcohols and polyoxyethylene sorbitol ethers, preservatives, flavour additives, such as, for example, peppermint oil or natural sweeteners or saccharin or other artificial sweeteners and the like, can likewise be added.

The dosage unit formulations for oral administration can, if desired, be encapsulated in microcapsules. The formulation can also be prepared in such a way that the release is extended or retarded, such as, for example, by coating or embedding of particulate material in polymers, wax and the like.

Pharmaceutical formulations adapted for parenteral administration include aqueous and non-aqueous sterile injection solutions comprising antioxidants, buffers, bacteriostatics and solutes, by means of which the formulation is rendered isotonic with the blood of the recipient to be treated; and aqueous and non-aqueous sterile suspensions, which may comprise suspension media and thickeners. The formulations can be administered in single-dose or multidose containers, for example sealed ampoules and vials, and stored in the freeze-dried (lyophilised) state, so that only the addition of the sterile carrier liquid, for example water for injection purposes, immediately before use is necessary.

The individual agents that comprise the combinations can be administered to the patient at the same time or at different times depending upon their bioavailability and toxicity. Their packaging into kits for administration to the patient also forms part of this invention. The agents can be formulated in a single pharmaceutical composition or can be separately formulated.

Pharmaceutical compositions of the above combinations are used to treat patients having cancer. Vehicles for delivering the compounds of the present invention to target tissues throughout the human body include saline and D5W (5% dextrose and water). Excipients used for the preparation of oral dosage forms of the compounds of the present

invention include additives such as a buffer, solubilizer, suspending agent, emulsifying agent, viscosity controlling agent, flavor, lactose filler, antioxidant, preservative or dye. There are conventionally preferred excipients for parenteral and other administration. These excipients include serum albumin, glutamic or aspartic acid, phospholipids and fatty acids.

Formulations can be in liquid form stored in a vial or an intravenous bag. The compounds of the present invention may also be formulated in solid or semisolid form, for example pills, tablets, creams, ointments, powders, emulsions, gelatin capsules, capsules, suppositories, gels or membranes.

The preferred route of administration is intravenous. Other acceptable routes of administration include oral, topical, rectal, parenteral (injectable), local, inhalant and epidural administration. The compositions of the invention may also be conjugated to transport molecules or included in transport modalities such as vesicles, micelles, and polymers to facilitate transport of the molecules. Methods for the preparation of pharmaceutically acceptable compositions that can be administered to patients are known in the art.

The compositions of the invention may also be conjugated to transport molecules, monoclonal antibodies or transport modalities such as vesicles and micelles that preferentially target cancer cells or that potentiate cancer cells to receive drugs.

Pharmaceutical compositions including the compounds of the present invention can be administered to humans or animals. Dosages to be administered also conventionally depend on individual patient condition, indication of the drug, physical and chemical stability of the drug, toxicity, the desired effect and on the chosen route of administration (Robert Rakel, ed., Conn's Current Therapy (1995, W.B. Saunders Company, USA)).

Excipients can also include components such as micelles, vesicles and liposomes that enhance the therapeutic performance of the compound and other agents. The action of vesicles, micelles and liposomes includes improving the solubilization of the compounds and

agents, improving their delivery to tumour cells, and interacting with tumour cells to make these cells more permeable to compounds and agents. Improving efficiency could improve treatment or allow equivalent results with reduced dosing and side-effects.

Typical doses for each of the agents for use in this invention are in the normal ranges conventionally known for each known agent used individually to treat cancer. For pentamidine, typical doses are 2-8 mg/kg body weight in humans. These amounts can be lowered per this invention due to synergistic effects in the combinations. Typical dose ranges for each agent in the combinations are: pentamidine 2-8 mg/kg body weight in humans; gemcitabine 800-1250 mg/m^{2}of surface area in humans; CPT 11 75-350 mg/m^2 of surface area in humans; and oxaliplatin 85-130 mg/m^{2}of surface area in humans. Doses can be lowered from the amounts in these ranges typically by 10 to 50% due to synergism.

Regimens (e.g. timing of doses, durations, etc.) are conventionally determinable with the guidance of conventional usage of these agents individually.

In the case of pentamidine, for example, guidance may be obtained from a study of patients given 180 to 200 mg of pentamidine in a 2-hour infusion. It showed that levels in the bloodstream go down rapidly over a few hours; and that the kidneys excrete only 7 mg of pentamidine into the urine in the first 24 hours (Conte, J.E., Jr.: *J. Infect. Diseases* (1991), **163**, 169). Since pentamidine is not readily metabolized in the liver, almost all of the material is distributed from the blood stream to body tissue where it stays. In addition, the amount found in the urine does not increase significantly with repeated dosing. This means that when pentamidine is given repeatedly, it accumulates in body tissues. Pentamidine was detected in tissue 25 days after final dose. Hence, pentamidine is only slowly released from tissue. It is also widely distributed in tissue (Goa, K.L., Campoli-Richards, D.M.;*Drugs* (1987), **33**, 242). Thus, pentamidine can be administered to the patient before, after, or

concurrently with other chemotherapy since its effectiveness depends on its distribution to and persistence in body tissues over long periods.

The way in which other chemotherapy agents are used in conjunction with pentamidine depends on their pharmacological characteristics. Thus, a convenient mode of dosing is to take the normal cycle of administration of a chemotherapy drug and to precede it with administration of pentamidine. This may be illustrated in conjunction with the combination of, for example, cis-platinum used effectively in combination with pentamidine to control cancer growth. Cis-platinum reacts slowly with water in the body to give an active form that binds to tissue. If it is injected slowly into patients urinary excretion can be as high as 75%. Therefore, rapid dosing is often used to ensure that the kidneys cannot excrete the drug before it is distributed to body tissue (Belt, R.J., Himmelstein, K.J., Patton, T.F., Bannister, S.J., Sternson, L.A., Repta, A.J., *Cancer Treatment Rep.* (1979), **63**, 1515). Thus, when pentamidine is used in conjunction with cis-platinum, a prudent approach is to give pentamidine to the patient a day before cis-platinum so that the kidneys are not over burdened by the administration of the two drugs. If two doses of pentamidine are needed, the first can be given two days before cis-platinum (day -2) and the second can be given one day before cisplatinum (day -1).

Often in oncology combinations of drugs are used. In colon cancer, for instance, examples include the administration of oxaliplatin, 5-fluorourocil, and leucovrin "FOLFOX" or irinotican, 5-fluorouracil and leucovorin "FOLFIRI". These combinations are typically administered to the patient every two weeks. Thus, when pentamidine is added to therapy, it can conveniently be given one or two days before standard chemotherapy. However, since pentamidine persists in body tissue, it can be as effective if given several days before standard chemotherapy.

A further example relates to human pancreatic cancer. Here, a typical treatment cycle involves administration of gemcitabine 800-1250 mg/m^{2}of surface area once a week for three weeks followed by a week of rest. When pentamidine is used together with gemcitabine it can conveniently be administered during the first week of the cycle on day -2 and day -1 prior to the administration of gemcitabine.

As a further example of suitable pentamidine dosing for use in combination with other cancer regimens, pentamidine can be given to patients intravenously in the following doses prior to such chemotherapy:

	Day -2	Day -1
	Dose (mg/kg)	Dose (mg/kg)
Option 1		4
Option 2	4	4
Option 3		5
Option 4	5	5
Option 5		6
Option 6	6	6

Moreover, dosing for a patient can be either escalated from lower to higher options or reduced in, respectively, the absence or presence of side-effects and, as is conventional, following the advice of the treating physician. Because pentamidine accumulates in body tissue, as discussed, it can be administered at any time in the cycle of normal chemotherapy, i.e., dosing is not limited to day-1 and day-2. Optimal dosing can be routinely determined.

Since pentamidine has a side-effect profile and mechanism of action that is quite different to those of standard anticancer agents, it can be used in combination with them without inducing adverse drug reactions that are substantially worse than those induced by the drugs when used alone. Given the life-threatening nature of many cancers, patients are treated aggressively with chemotherapy. Treatment in conjunction with pentamidine can be given until side-effects of the standard chemotherapy agent become evident. At this point, administration of the standard chemotherapy agent can be halted and therapy with

pentamidine alone can be continued. The sustained use of pentamidine can be of benefit to patients since pentamidine is an effective anticancer agent in its own right. Reasonable doses of pentamidine to be used either in combination therapies or in mono-therapy are 6 mg/kg of body weight or 4 mg/kg of body weight.

Pentamidine has side-effects of its own, the most significant of which in this context is the possibility that patients might suffer pancreatitis. This side-effect can be pronounced if pentamidine is administered for many consecutive days, e.g., 10 to 15 at doses of 4-6 mg/kg/day as is the case when it is used to treat parasitic diseases. However, in the dosing schedules described herein where one or two doses may be given every two weeks, the risks of pancreatitis are greatly reduced. If pancreatitis occurs, pentamdine administration can be stopped until the patient recovers but standard chemotherapy may be continued in the interim. Sustained use over many days with lower doses of pentamidine, e.g., 1-4 mg/kg per day, affords another means of reducing toxicity while maintaining efficacy.

As in all therapies, treating physicians have to consider the characteristics and use of drugs in light of the patients' physical condition and symptoms and administration has to be routinely modulated accordingly.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The preceding preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The entire disclosure[s] of all applications, patents and publications, cited herein are incorporated by reference herein.

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

EXAMPLES

Example 1

Purpose:

Synergistic effect in anticancer therapy was generated by using pentamidine in combinations with each of the following: taxol, oxaliplatin, gemcitabine, or CPT 11.

Method:

Cell Survival – MTT assay: The MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5 diphenyl tertrazolim bromide) method of determining cell growth/cytotoxicity was used to determine cell survival. MTT is a tetrazolium salt that binds to b mitochondrial dehydrogenases of living cells. Binding converts yellow, water soluble MTT to an insoluble, purple formazan crystal. The crystals are solubilized with a 50% N,N-dimethylformamide (vol/vol), 20% SDS (wt/vol) solution (pH4.7), and absorbance is determined at a wavelength of 570 nm. Unbound MTT is not detectable at this wavelength. The amount of bound MTT measured in the assay is proportional to the number of live cells present. (Niks and Otto 1990: "Towards an optimized MTT assay," J. Immunol. Methods. 130, 149-151, Hussain et al. 1993; "A new approach for measurement of cytotoxicity using colorimetric assay," J. Immunol. Methods. 160, 89-96).

Cells were harvested from cell cultures using the standard protocol (Trypsin/EDTA). The cells (1000 to 5000 cells in 50µl of solution depending on cell type used) were then plated and incubated overnight at 37°C before the addition of the agent or combination of agents.) After 2 days of incubation at 37°C, 10 µl of a 5 mg/ml solution of MTT was then added to all the wells and to a media control well. The plates were further incubated for 4 hours. A 100 µl of MTT solubilization buffer was added and the plates were incubated

overnight at 37°C. The plates were then read on the ELISA plate reader with absorbance at 570 nm and a reference at 630 nm.

The effects of combinations were tested on three representative cancer cell lines: H661 (lung cancer (carcinoma)), MCF-7 (breast cancer (adenocarcinoma, pleural effusion)), and HT29 (colon cancer (adenocarcinoma, primary tumor)). Initial assays were carried out to determine the concentrations at which taxol, oxaliplatin, gemcitabine, or CPT 11 (also known as irinotecan) killed approximately 10% of the cells under investigation. In a second series of assays, pentamidine was added to the cell cultures. Several concentrations of pentamidine were tested in combination with each of taxol, oxaliplatin, gemcitabine, or CPT 11 and the LC_{50} was determined i.e. the concentration of pentamidine that killed 50% of the remaining cells.

The addition of pentamidine to a sub-lethal dose of cytotoxic chemotherapeutic agents greatly increased the anticancer effect (from 2-fold to 50-fold) for breast cancer (MCF-7), lung cancer (H661) and colon cancer (H T29) cells, as shown in Table 1.

Table 1: LC_{50} of Pentamidine On Cancer Cells When Used Alone Or In Combination With Taxol, Oxaliplatin, Gemcitabine, or CPT 11

Cancer cell type	Pentamidine (mM) 2 days[1]	Pentamidine (mM) with Taxol (2 μM)	Pentamidine (mM) With Oxaliplatin (0.25 μM)	Pentamidine (mM) With CPT11 (0.25 μM)	Pentamidine (mM) With Gemcitabine (0.26 μM)
H661	0.15	0.007	0.003	0.003	0.027
MCF-7	0.15	0.06	0.009	0.026	0.066
HT29	0.27	0.13	0.009	0.012	0.030

[1] *Length of exposure to mixture.*

Since the concentration of the cytotoxic agents killed 10% of the cells when used alone, an additive effect would simply have manifested itself as a small improvement in the performance of pentamidine, roughly corresponding to an improvement of about 10% versus that of pentamidine alone. The data show that the combinations allowed the concentration of pentamidine to be reduced by 100% (HT29 with taxol - worst case examined) and by 5000% (oxaliplatin or gemcitabine with H661 – best cases examined) while maintaining the same cell killing efficiency. All the cytotoxic agents displayed a strong synergistic effect when used in combination with pentamidine.

This effect is also demonstrated by the data in Tables 2A - C, where pentamidine and various cytotoxic agents are used at higher concentrations than those described above. The extent to which each kill cells when used alone is reported in the tables. The data are followed by the extent of cell death when the compounds are used in combination. Again the combinations show synergy and not simple additivity.

Table 2A: Percentage of cells killed at various drug concentrations

Cell line	**Pentamidine (0.15 mM)**	**CPT-11 (3.2 μM)**	**Pentamidine (0.15 mM) + CPT-11 (3.2μM)**
H661	50%	14%	93%
MCF7	50%	30%	100%
HT29	13%	6%	90%

Table 2B

Cell line	**Pentamidine (0.15 mM)**	**Oxaliplatin (0.25 μM)**	**Pentamidine (0.15 mM) +**

			Oxaliplatin (0.25 μM)
H661	50%	14%	95%
MCF7	50%	8%	71%
HT29	13%	10%	93%

Table 2C

Cell line	Pentamidine (0.15 mM)	Gemcitabine (0.26 μM)	Pentamidine (0.15 mM) + Gemcitabine (0.26 μM)
H661	50%	25%	100%
MCF7	50%	23%	100%
HT29	13%	23%	100%

Example 2 – Clinical Trial Pancreatic Cancer

A non-randomized, open label, Phase I/IIa clinical trial is designed to assess the effect of intravenous (I.V.) pentamidine for subjects with advanced or metastatic pancreatic cancer undergoing standard chemotherapy (gemcitabine regimen).

A total of 15-20 subjects with pancreatic cancers are being enrolled over a period of 12 months. Pentamidine is being administered I.V. over a period of 1-2 hours, in a continuous regimen, with a starting dose of 6 mg/kg of body weight of pentamidine isethionate. Pentamidine is being administered two days prior (Day -2) to the start of a 21-28 day standard chemotherapy cycle for pancreatic cancer. A further dose is being given on Day -1.

All subjects are being given a standard of care chemotherapy regimen. Subjects continue treatment as long as they receive clinical benefit, or until objective disease progression is documented, or until they withdraw from the trial for other reasons.

Example 3 – Clinical Trial Colon Cancer

A non-randomized, open label, Phase I/IIa clinical trial is designed to assess the effect of I.V. pentamidine for subjects with metastatic colon cancer undergoing second-line chemotherapy (modified FOLFOX-6 (mFOLFOX6), or Capecitabine and Oxaliplatin, or FOLFIRI or IROX, or Capecitabine and Irinotecan containing regimens) treatment and/or chemotherapy as per physician choice for third line and above treatment regimen. (FOLFOX regimens contain oxaliplatin, FOLFIRI regimens contain CPT 11 also known as Irinotecan; and IROX regimens contain Irinotecan and Oxaliplatin). Patients may also receive bevacizumab (Avastin) as part of chemotherapy or cetuximab (Erbitux) or panitumumab (Vectibix). Twenty-two patients are enrolled to date.

Pentamidine is being administered two days prior (Day -2) to the start of a 14 day standard chemotherapy cycle for metastatic colon cancer. A further dose is being given on Day -1. Pentamidine is being administered I.V. over a period of 1-2 hours, in a continuous regimen, with a starting dose of 4 mg/kg of body weight of pentamidine isethionate.

The study design allows for dose escalation to 6 mg/kg of pentamidine and for continuing patients on pentamidine alone when side effects from the other anticancer agents become pronounced. Both dose escalation and treatment with pentamidine alone are at the discretion of the treating physician.

The following combinations with pentamidine are tested in patients: FOLFOX (fluorouracil, folinic acid and oxaliplatin) or modified versions thereof with or without bevacizumab, FOLFIRI (fluorouracil, folinic acid and irinotecan) or modified version thereof with or without bevacizumab, CPT-11 with or without bevacizumab, CPT-11 with or without oxaliplatin, and capecitabine. Almost all patients will have previously failed on their current treatment or a combination thereof.

Interim results demonstrate that pentamidine significantly enhances overall survival when compared with best current therapy.

Example 4 - Clinical Trial Breast and Ovarian Cancer

A non-randomized, open label, Phase I/IIa clinical trial is designed to assess the effect of I.V. pentamidine for subjects with breast and/or ovarian tumors and/or metastases derived from breast and/or ovarian tumours. Patients are receiving pentamidine beginning with two doses of pentamidine isethionate (6 mg/kg) prior to each cycle of standard chemotherapy.

Pentamidine is being administered two days prior (Day -2) to the start of a standard chemotherapy cycle for breast and/or ovarian cancer. A further dose is being given on Day -1. Pentamidine is being administered I.V. over a period of 1-2 hours, in a continuous regimen.

For the treatment of localized or metastatic breast or ovarian cancer, doxorubicin, 5-fluorouracil, carboplatin, and paclitaxel are examples of components of standard chemotherapy regimens. Capecitabine (Xeloda®), an orally administered systemic prodrug of 5'-deoxy-5-fluorouridine (5'DFUR) which is converted to 5-fluorouracil, is also used. The incorporation of pentamidine in combination with standard chemotherapy, for example,

doxorubincin or 5-fluorouracil or carboplain or paclitaxel, comprises another aspect of this invention.

The study design allows for continuing patients on pentamidine alone when side effects from the other anticancer agents become pronounced. Dose escalation, reduction, and treatment with pentamidine alone are at the discretion of the treating physician.

Example 5 - Phase I/II trial of pentamidine with fluorouracil, oxaliplatin and/or CPT-11 containing chemotherapy in patients with previously treated metastatic colorectal cancer (mCRC)

INTRODUCTION

- Colorectal cancer is the 2nd leading cause of cancer death in North America
- Combination chemotherapy with biologic agents has extended median survival in patients (pts) with mCRC to approximately 24 months
- Novel agents are being actively investigated
- Endo-exonuclease (EE), a key enzyme in DNA recombination and repair, has been show to be overexpressed in cancer cells[1,2,3]
- Pentamidine inhibits EE and has been shown to have disease stabilizing activity in metastatic cancer [1,4]
- *In vitro* studies have shown that pentamidine can potentiate the effects of cytotoxic chemotherapy on malignant cells; by impairing their capacity for DNA repair, they are more susceptible to DNA damaging agents [1,2,3]

STUDY OBJECTIVES

- To evaluate the safety and efficacy of combining pentamidine with fluoropyrimidine, oxaliplatin and/or CPT-11 containing chemotherapy (CTX) in pts with mCRC who have failed prior lines of standard treatment
- Primary endpoints: treatment safety and tolerability
- Secondary objectives: response rate (RR), progression-free survival (PFS) and overall survival (OS)

METHODS

- Eligibility criteria: radiologic evidence of progression of mCRC on ≥ 1 prior lines of standard CTX; ≥18y/o; ECOG 0-2; normal EKG; adequate hematologic, hepatic and renal function; life expectancy > 3 months; informed consent
- Pentamidine at 4mg/kg was begun the day before CTX and gradually escalated to a maximum dose of 6 mg/kg for 2 consecutive days before CTX (see Figure 1)
- CTX was chosen by the patient's treating oncologist
- Adverse events (AEs) were graded according to the NCI CTCAEv3 classification system
- Dose limiting toxicity (DLT) = any grade 3 or 4 occurring within the first 2 cycles of treatment that can be attributed to pentamidine
- Maximum dose of pentamidine chosen for this study was 6 mg/kg for 2 consecutive days before CTX; higher doses were not tested
- Screening CT chest/abdomen/pelvis within 28 days of starting study treatment -> repeat q3 cycles or as per standard of care
- Radiologic response was assessed according to RECIST criteria
- An extension phase was opened for pts with no disease progression after 6 cycles of pentamidine

RESULTS: Clinical Characteristics

- Preliminary results on the initial 17 patients enrolled in this ongoing phase I/II trial are presented (Table 3)
- Median age of first 17 pts = 66 (range 43-82)
- Median treatment duration of first 17 pts = 15 weeks (range 0.3-58)

Median patient age	66 years (43-82)	-
Male : Female ratio	10:7	59%:41%
ECOG status:		
0	10	59%
1	5	29%
2	2	12%
Number of previously failed CTX lines:		
1	3	18%
2	7	41%
3 or more	6	35%
Data pending	1	6%
Current CTX (selected by treating oncologist)		
Irinotecan-based (with Bevacizumab)	8 (2)	47% (12%)

Oxaliplatin-based (with Bevacizumab)	4 (1)	24% (6%)
Irinotecan and Oxaliplatin-based	4 (1)	24% (6%)
Other	1	6%
Initial dose of pentamidine prior to CTX:		
1 x 4mg/kg	3	18%
2 x 4mg/kg	12**	71%**
2 x 6mg/kg	2	12%

Note: A failed line of CTX = disease progression during or within 6 months of CTX end. Failure of CTX due to toxicity was not counted as CTX failure in this study. Numbers may not add up to 100% due to rounding.
* Study and data collection have not closed. ** One patient was mistakenly administered 5mg/kg instead of 4mg for the first six weeks (3 cycles) of treatment.

RESULTS: Adverse Events

- 13 out of 17 pts were evaluable for preliminary safety and tolerability analysis (Table 4). Data pending for 4 pts
- Grade 3/4 AEs attributed to pentamidine were hyperglycaemia (23%) and hyperlipasemia (15%).
  - NB. Concomitant drugs (e.g. decadron), CTX preparation in D5W and/or inclusion of pts with type 2 diabetes may confound direct attribution of hyperglycaemia to pentamidine
- DLT were anorexia and hyperglycaemia, which each occurred in 8% of pts
- **Toxicity was consistent with known side effects of pentamidine**

CNS:							
Fainting/syncope	8	3	18	4mg/kg x 1	No	no	Tx delayed
Constitutional:							
Fatigue	5	4	4	4mg/kg x 2	No	no	discontinued study
	7	3	14	4mg/kg x 1	possibly	possibly	continued study
General deterioration	5	4	4	4mg/kg x 2	No	no	discontinued study
Dermatologic:							
Hand-foot reaction	3	3	2	4mg/kg x 2	No	definitely	Tx delayed

Gastrointestinal:							
Anorexia	5	3	2	4mg/kg x 2	possibly	possibly	continued study
Diarrhea	5	3	3	4mg/kg x 2	No	definitely	continued study
	10	3	5	4mg/kg x 2	possibly	possibly	Tx delayed
	12	3	3	4mg/kg x 2	No	probably	continued study
Genitourinary :							
Urinary tract infection	11	3	2	4mg/kg x 2	No	no	Tx delayed
Hematologic:							
Neutropenia	1	3	5	4mg/kg x 1	possibly	definitely	continued study
Metabolic:							
Hyperglycaemia	1	3	1	4mg/kg x 1	possibly	no	continued study
	11	4	8	4mg/kg x 2	definitely	no	discontinued study
	12	3	10	4mg/kg x 2	definitely	possibly	discontinued study
Elevated Lipase	7	3	14	4mg/kg x 1	possibly	possibly	Tx delayed
	8	3	19	4mg/kg x 1	definitely	no	discontinued study

Note: Data pending on 4 pts. Grey text = adverse events not attributed to pentamidine. Tx = therapy. * Study and data collection have not closed.

RESULTS: Clinical Outcomes

- 14 out of 17 patients were evaluable for response (Table 5)
- 35% of pts had SD and 47% had PD at patient exit
- Preliminary analysis of the median PFS time = 4.4 months (Figure 2)
- Median OS time has not yet been reached
- Changes in CEA did not correlate with response (data not shown)

Median duration of treatment	15.1 wk (0.3-58.4)	-
Best response during treatment*:		
CR	0	0%
PR	1	6%
SD	10	59%
PD	4	24%
Unevaluable (Data pending)	3 (1)	18% (6%)
Response at patient exit from trial**:		

CR	0	0%
PR	0	0%
SD	6	35%
PD	8	47%
Unevaluable (Data pending)	3 (1)	18% (6%)
Reasons for patient exiting the trial:		
Toxicity attributed to pentamidine	3	18%
PD	6	35%
Fatigue	2	12%
Other (general deterioration, surgery, jaundice)	3	18%
Data pending	3	18%

Note: CR = complete regression, PR = partial regression, SD = stable disease, PD = progressive disease. Numbers may not add up to 100% due to rounding. * Study and data collection have not closed. ** Best response indicates best tumour response achieved as determined by CT scan.

CONCLUSIONS

- Toxicity associated with the combination of pentamidine and CTX was consistent with that observed in the literature and was manageable
- Pentamidine & CTX appears to have disease stabilizing activity in mCRC that has progressed on standard lines of treatment

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From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention and, without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

Example 6 – Human Xenograph Study

A human xenograph study in a mouse model was conducted to demonstrate the anti tumor activity of Pentamidine administered intraperitoneally twice a week in combination with Gemcitabine administered intraperitoneally twice a week in the BxPC3 human pancreas xeongraft model on CB17 SCID female mice.

BxPC3 cells were transplanted subcutaneously into the flank of each animal as a suspension of tumor cells (5×10^6 cells in 0.1 mL in PBS) on January 4, 2010 (day 1). Transplantation was performed under a laminar airflow hood. Four (4) days after BxPC3 cell injections, mice were randomized (day treatment began) into 4 groups of 10 mice each based on tumor size so that the average tumor size in each group was comparable. Five (5) mice were rejected of this study because no tumor grew, tumors were too small, or tumors were too big. Animals were labeled using the "ear punching" method so that for each group, all 10 animals could be distinguished. Each group of 10 mice was housed in 2 separate cages of 5 mice each; animal numbers 1 to 5 were housed in Cage A and animal numbers 6 to 10 were housed in Cage B.

Prior to every dosing injection, each animal was weighed and received their respective formulations. Mice in group 1 were treated intraperitoneally for two consecutive days, stop one day and two other consecutive days for nine weeks (one mouse reached one end points) by direct injection in the abdominal cavity with 0.9% NaCl *usp*. Mice in group 2 were

treated intraperitoneally, bi-weekly (Monday and Thursday) at 45 mg/kg with Pentamidine for nine weeks. Mice in group 3 were treated intraperitoneally, bi-weekly (Tuesday –Friday) at 150 mg/kg with Gemcitabine for eleven weeks. Mice in group 4 were treated first with Pentamidine administerd intraperitoneally bi-weekly (Monday and Thursday) at 45 mg/kg and with Gemcitabine administerd intraperitoneally bi-weekly (Tuesday-Friday) at 150 mg/kg for twelve weeks as described in Table 6. The dose volume was 30 mL/kg for mice treated intraperitoneally.

Table 6: Treatment Groups

Group	Compound	Route	Dose (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Treatment Frequency
1	0.9% NaCl *usp*	IP	0	30	0	Two consecutive days, stop one day and two other consecutive days for nine weeks
2	**Pentamidine**	IP	45	30	1.5	Bi-weekly Monday and Friday for nine weeks
3	**Gemcitabine**	IP	150	30	5	Bi-weekly Tuesday and Friday for eleven weeks
4	**Pentamidine**	IP	45	30	1.5	Bi-weekly Monday and Thursday for twelve weeks
	Gemcitabine	IP	150	30	5	Bi-weekly Tuesday and Friday for twelve weeks

IP = Intra peritoneally
mg/kg = milligram.kilogram
mL/kg = milliliter per kilogram
mg/mL = milligram per milliliter

At termination, when the tumor volume of one of the mice in the group reached 1500 mm^3, the whole group was sacrificed, and animals were anaesthetized using isoflurane and sacrificed by cervical dislocation.

All treatments were well-tolerated. The results are shown in Figure 3.

To a high degree of statistical confidence, use of pentamidine alone and of pentamidine and gemcitabine in combination were found to have a beneficial effect in the treatment of pancreatic cancer.

WHAT IS CLAIMED IS:

1. A method of inhibiting the proliferation of cancer cells comprising administering to a patient in need thereof (1) pentamidine and (2) oxaliplatin, gemcitabine, taxol, 5-fluorouracil or CPT 11.

2. A composition comprising (1) pentamidine and (2) oxaliplatin, gemcitabine, taxol, 5-fluorouracil or CPT 11.

3. A method of claim 1 wherein the amounts of pentamidine and oxaliplatin, gemcitabine, taxol, 5-fluorouracil or CPT 11 are synergistic.

4. A method of claim 1 wherein the cancer cells are squamous cell carcinoma cells, large cell carcinoma of lymph node cells, breast cancer cells, colon cancer cells, lung carcinoma cells, melanoma cells, pancreatic cancer cells, leukemia cells, non-small cell lung cancer cells, CNS cancer cells, ovarian cancer cells, renal cancer cells or prostate cancer cells.

5. A method of claim 1 wherein the cancer cells are pancreatic cancer cells.

6. A method of claim 1 wherein pentamidine and oxaliplatin are administered.

7. A method of claim 1 wherein pentamidine and gemcitabine are administered.

8. A method of claim 1 wherein pentamidine and CPT 11 are administered.

9. A method of claim 1 wherein pentamidine and taxol are administered.

10. A method of claim 7 wherein the cancer is localized or metastatic pancreatic cancer.

11. A method of claim 9 wherein the cancer is localized or metastatic breast cancer.

12. A method of claim 6 where the cancer is localized or metastatic colon cancer.

13. A method of claim 8 where the cancer is localized or metastatic colon cancer.

14. A method of treating cancer in a patient comprising administering to said patient (1) pentamidine and (2) oxaliplatin, gemcitabine, taxol, 5-fluorouracil or CPT 11, optionally in each case with the further administration of folinic acid, fluorouracil, bevacizumab, cetuximab, panitumumab or a combination thereof.

15. A method of treating pancreatic cancer in a patient comprising administering pentamidine to the patient.

16. A method of claim 15 wherein the cancer is localized or metastatic pancreatic cancer.

17. A method of treating localized or metastatic, pancreatic, breast, ovarian or colon cancer in a patient comprising administering pentamidine to the patient in combination with standard chemotherapy for pancreatic, breast, ovarian or colon cancer, respectively.

18. A method of treating ovarian cancer in a patient comprising administering pentamidine to the patient.

19. A method of claim 18 wherein the cancer is localized or metastatic ovarian cancer.

20. A method of treating breast cancer in a patient comprising administering pentamidine to the patient.

21. A method of claim 18 wherein the cancer is localized or metastatic breast cancer.

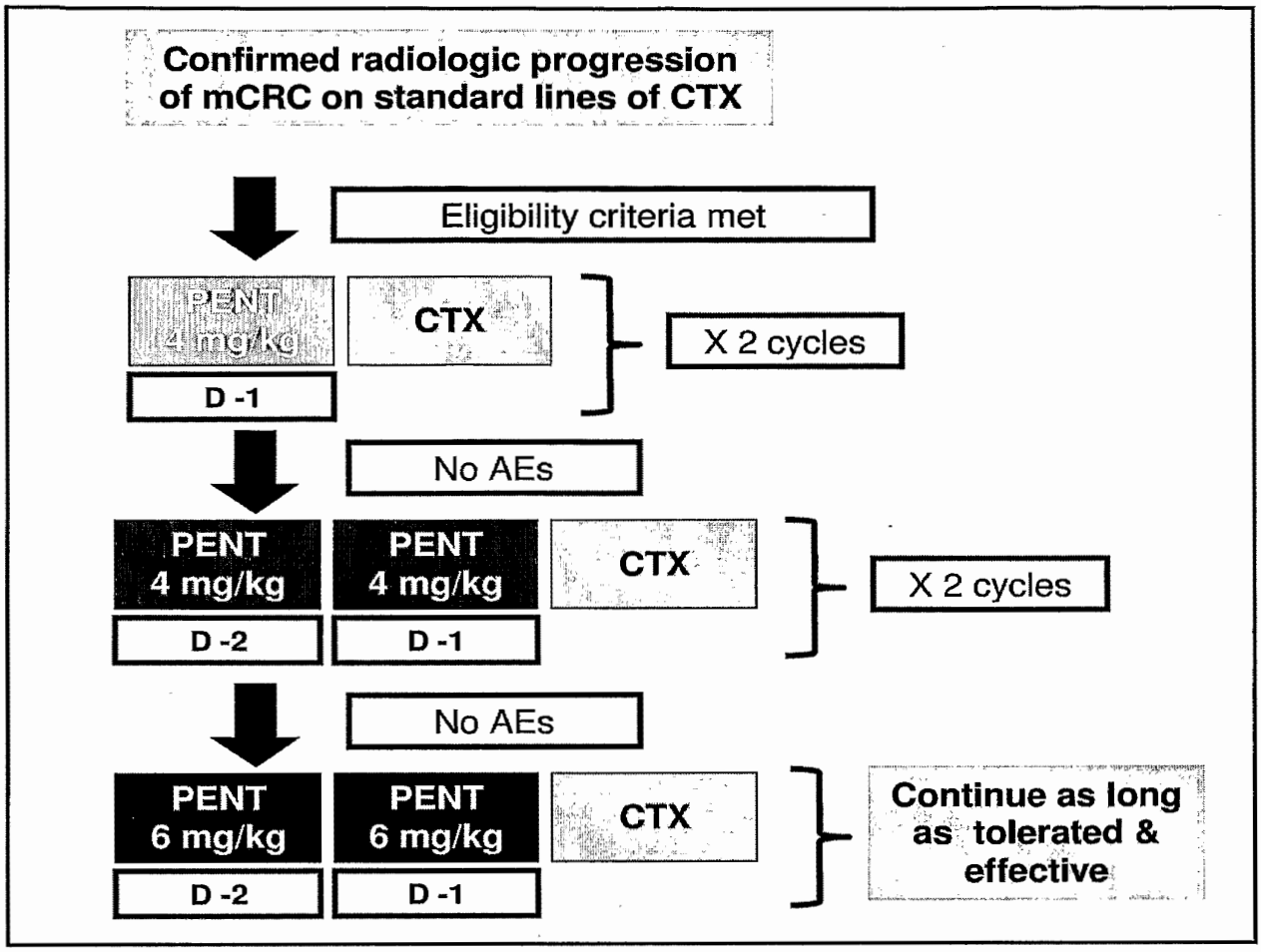
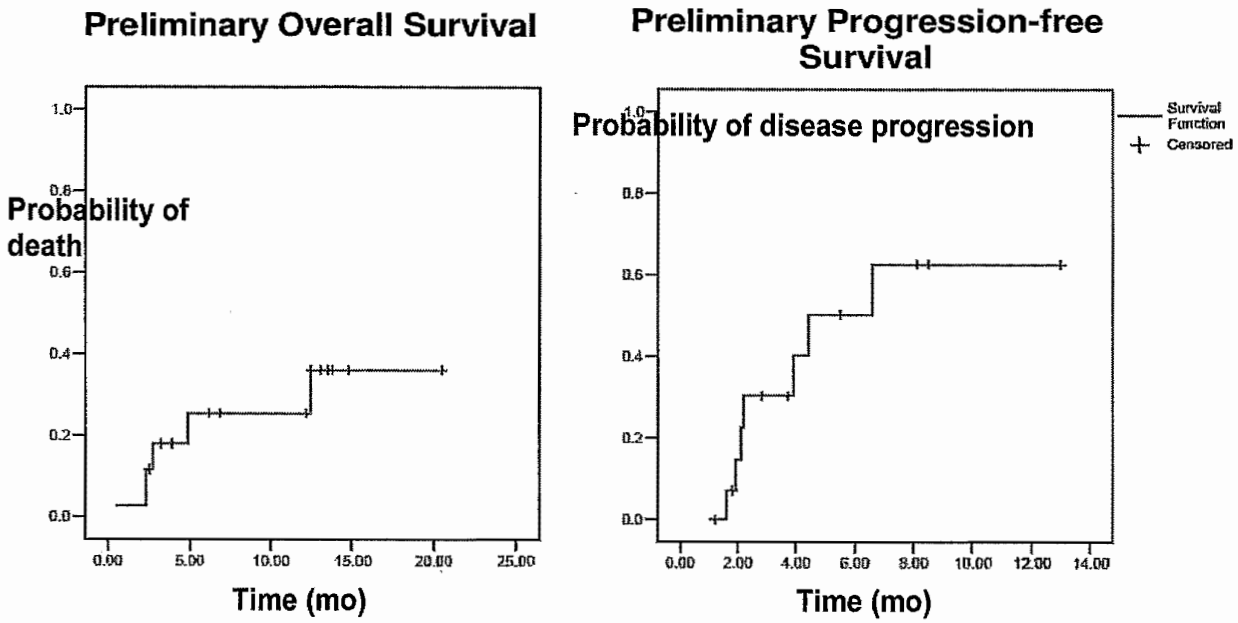

Confirmed radiologic progression of mCRC on standard lines of CTX
Eligibility criteria met
PENT 4 mg/kg
CTX
D -1
X 2 cycles
No AEs
PENT 4 mg/kg
PENT 4 mg/kg
CTX
D -2
D -1
X 2 cycles
No AEs
PENT 6 mg/kg
PENT 6 mg/kg
CTX
D -2
D -1
Continue as long as tolerated & effective

Figure 1

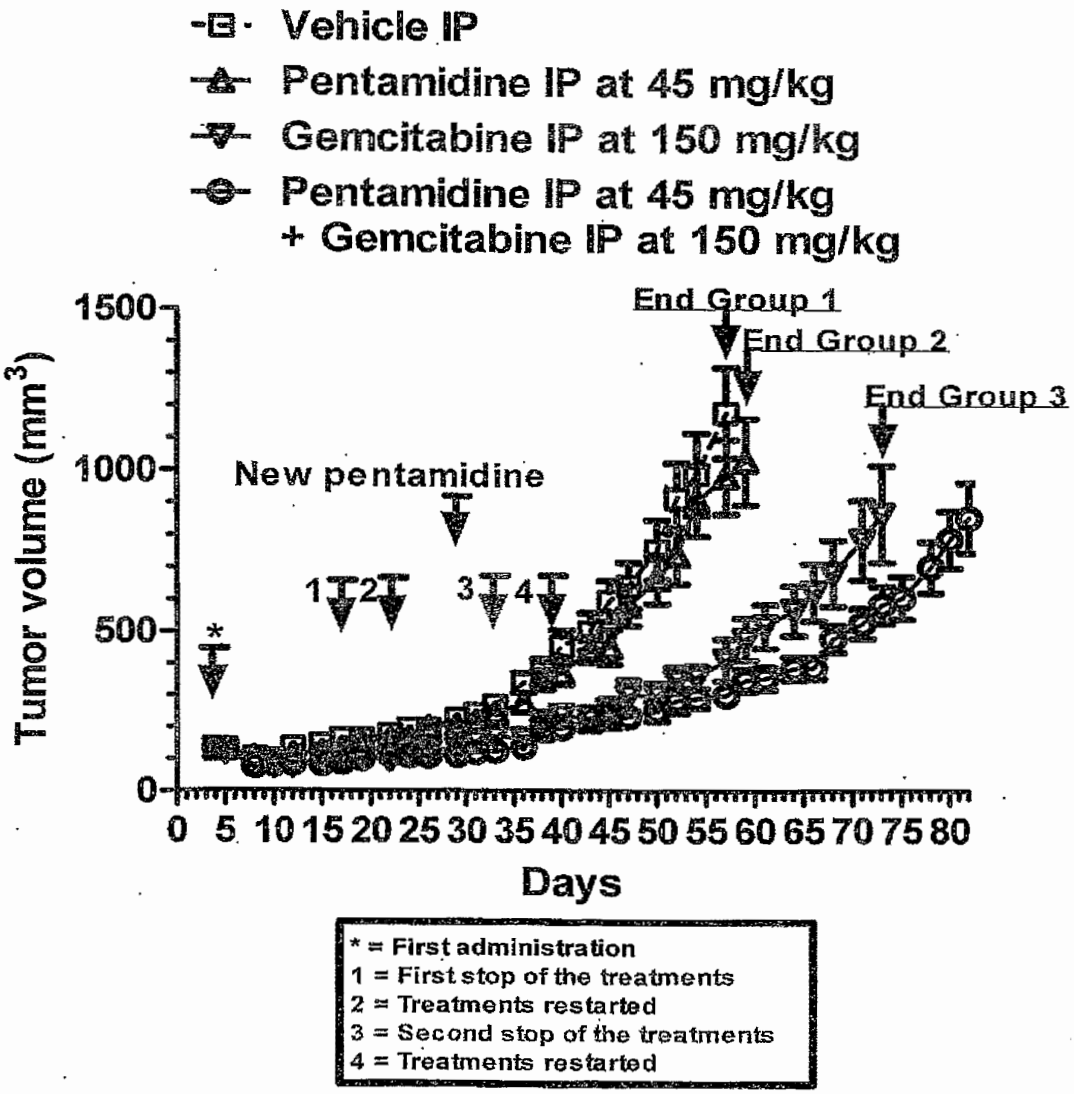
Figure 2: Survival Curves*



* Study and data collection have not closed.

Figure 3

Effect of Pentamidine in combination with Gemcitabine on tumor size (mm^3) over time



(12) 按照专利合作条约所公布的国际申请

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(81) 指定国 (除另有指明，要求每一种可提供的国家保护): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, 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, PE, 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。

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— 包括国际检索报告(条约第 21 条(3))。

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(54) **Title**: LIPOSOME OF IRINOTECAN OR ITS HYDROCHLORIDE AND PREPARATION METHOD THEREOF

(54) **发明名称** : 伊立替康或盐酸伊立替康脂质体及其制备方法

(57) **Abstract**: A liposome of irinotecan or its hydrochloride and its preparation method are disclosed. The liposome comprises irinotecan or its hydrochloride, neutral phospholipid and cholesterol, wherein the weight ratio of cholesterol to neutral phospholipid is 1:3-5. Said liposome is prepared by ion gradient method.

(57) **摘要**:

一种伊立替康或盐酸伊立替康脂质体及其制备方法。脂质体含有伊立替康或盐酸伊立替康、中性磷脂以及胆固醇，胆固醇与中性磷脂的重量比例为 1：3—5，其通过离子梯度法制备得到。

伊立替康或盐酸伊立替康脂质体及其制备方法

技术领域

本发明涉及一种伊立替康或盐酸伊立替康脂质体及其制备方法，以及含有该脂质体的注射剂及其制备方法。

背景技术

伊立替康是喜树碱的半合成衍生物。喜树碱可特异性地与拓扑异构酶 I 结合，后者诱导可逆性单链断裂，从而使 DNA 双链结构解旋；伊立替康及其活性代谢物 SN-38 可与拓扑异构酶 I-DNA 复合物结合，从而阻止断裂单链的再连接。现有研究提示，伊立替康的细胞毒作用归因于 DNA 合成过程中，复制酶与拓扑异构酶 I-DNA-伊立替康（或 SN-38）三联复合物相互作用，从而引起 DNA 双链断裂。

盐酸伊立替康药理作用明显，临床疗效确切，在恶性肿瘤治疗中应用广泛，但是，它和其他喜树碱类药物一样，同样存在的一个问题就是，其结构中的饱和内酯环具有 pH 依赖性，在生理条件下会可逆的转变为其羧酸盐形式，而使抗肿瘤活性减弱。盐酸伊立替康的现有市售制剂为盐酸伊立替康注射液及其冻干粉针制剂，临床上经静脉给药后，游离药物直接处在偏碱性的生理环境下，其结构中的内酯环易发生水解反应转变为羧酸盐形式，从而失去活性，间接的降低了药物的疗效。而且，制剂的毒副作用较大，主要表现为中性粒细胞减少和迟发性腹泻。

脂质体作为近年来研究较为广泛的一种药物载体，其主要特点是可以保护被包封药物，增加药物稳定性，改变药物在体内的分布行为，携载药物被动或主动靶向到病变部位。脂质体作为抗肿瘤药物的良好载体可有效的提高药物疗效，降低毒副作用。

国际申请 WO2005/117878 公开了伊立替康脂质体的处方和制备方法，该制剂中含有伊立替康或盐酸伊立替康、选自氢化大豆磷脂酰胆碱、磷脂酰乙醇胺、卵磷脂、心磷脂等磷脂、胆固醇。同样,中国专利申请 CN1994279A 也公开了伊立替康脂质体的处方和制备方法，其

中实施例 5 的使用磷脂酰胆碱作为磷脂制备了脂质体。

虽然上述专利文献中记载的处方已经具有较好效果，但是如果制备成适合人体使用的制剂，该脂质体在稳定性、粒径等方面仍不能令人满意。

发明内容

本发明的目的是提供一种载药量较高，且能同时满足包封率高，稳定性好、适合制备成制剂的伊立替康或盐酸伊立替康脂质体。

虽然目前一些文献（例如国际申请 WO2005/117878 和 CN1994279A）中记载了伊立替康的脂质体组成和制备方法，其中个别方案的部分指标较好。但针对稳定性、粒径等方面的控制并未提供任何信息。申请人经对脂质体进一步研究，令人惊奇的发现当选择处方的辅料和用量关系符合某些条件时，特别胆固醇的用量对脂质体粒径和稳定性有一定的影响，在选择中性磷和胆固醇的基础上控制二者之间的比例，能够使脂质体的粒径变得小而均匀，并且提高了脂质体的稳定性。与其他处方相比，本申请脂质体的稳定性等指标明显提高。另外本发明与国际申请 WO2005/117878 和 CN1994279A 等技术相比，产品中不含碱性官能团化合物和阳离子脂质，处方组成简单，载药量高，稳定性好，本发明的脂质体有很好的抗肿瘤效果。

本发明的脂质体含有伊立替康或盐酸伊立替康、中性磷脂以及胆固醇，其中所述胆固醇与中性磷脂的重量比例为 1：3~5，优选比例为 1：3.5~4.5，最优选为 1：4。

本发明所使用的中性磷脂可选择氢化大豆卵磷脂（HSPC）、蛋黄磷脂（EPC）、大豆磷脂（SPC）等材料，特别是当中性磷脂使用氢化大豆卵磷脂时，其效果最好。当进一步控制药物和磷脂之间的重量配比关系为下述关系时，脂质体的载药量极大地提高：

伊立替康或盐酸伊立替康	1 份
中性磷脂	2～5 份，优选 2.5-4 份。

本发明的脂质体可根据本领域常规的脂质体制备方法制备得到，对于本发明的脂质体来说，优选使用离子梯度法制备。在使用离子梯

度法时，所述脂质体内水相与外水相之间具有缓冲剂形成的离子梯度，优选所述脂质体内水相比外水相具有离子浓度高的离子梯度，这可提高贮存期间脂质体的粒径稳定，更好的维持药效，这能够控制脂质体平均粒径小而均匀，可使脂质体粒径在贮存期的变化减小到最低程度。

本发明通过在配方中加入亲水性高分子的脂质衍生物可使脂质体粒径在贮存期的变化减小到最低程度，同时聚乙二醇衍生物的加入可以延长脂质体在体内的循环时间。聚乙二醇衍生物选自聚乙二醇 2000-二硬酯酰磷脂酰乙醇胺（DSPE-PEG_{2000}）聚乙二醇 5000-二硬脂酰磷脂酰乙醇胺、聚乙二醇 2000-二棕榈酰磷脂酰乙醇胺、聚乙二醇 5000-二棕榈酰磷脂酰乙醇胺。因此为了提高药物的长效性，本申请优选在脂质体中添加亲水性高分子的脂质衍生物，在该处方比例基础上，使用 DSPE-PEG_{2000} 是效果最明显的。优选脂质衍生物与伊立替康或盐酸伊立替康的重量比为 0.2～0.4。

脂质体可进一步含有带电荷磷脂，带电荷磷脂选自二月桂酰磷脂酰甘油、二棕榈酰磷脂酰甘油、二硬脂酰磷脂酰甘油、二肉豆蔻酰磷脂酰甘油、二油酸磷脂酰丝氨酸、二油酰磷脂酰甘油、二月桂酰磷脂酸、二肉豆蔻酰磷脂酸或二硬脂酰磷脂酸中的一种或多种，且带电荷磷脂与中性磷脂的重量比例为 1:5～1:100。

本发明优选的脂质体含有以下重量配比的成分：

盐酸伊立替康	1 份
氢化大豆卵磷脂	3.4-3.8 份
聚乙二醇 2000-二硬酯酰磷脂酰乙醇胺	0.34-0.38 份
胆固醇	0.8-0.95 份，

且胆固醇与氢化大豆卵磷脂的比例为 1：4。

本申请还提供了伊立替康或盐酸伊立替康脂质体的制备方法，本发明的脂质体可选择通常的脂质体制备方法制备得到。本领域技术人员可根据本发明提供的脂质体的处方，选择各种方法制备得到。对于本发明的脂质体所选择的处方而言，优选的制备方法是离子梯度法。该制备方法包含下述步骤：

1)通过下述 A 至 D 中任何一种方法制备空白脂质体：

A、根据配方选用中性磷脂、胆固醇溶于无水乙醇或无水乙醇-叔丁醇混合溶剂中，与缓冲剂混合，减压除掉乙醇后得到空白脂质体粗品，之后采用高压均质机或/和挤压设备制备空白脂质体至所需的粒度；

B、根据配方选用中性磷脂、胆固醇溶于氯仿或氯仿-甲醇混合溶剂，旋转蒸发形成脂质膜，加入缓冲剂水化得到空白脂质体粗品，之后采用高压均质机或/和挤压设备制备空白脂质体至所需的粒度；

C、根据配方选用中性磷脂、胆固醇与缓冲剂混合，之后采用高压均质机或/和挤压设备制备空白脂质体至所需的粒度；

D、根据配方选用中性磷脂、胆固醇溶于无水乙醇或无水乙醇-叔丁醇混合溶剂中，与缓冲剂混合，之后采用高压均质机或/和挤压设备制备空白脂质体至所需的粒度；

2)脂质体膜内外水相离子梯度的产生：置换空白脂质体外水相，使脂质体内水相与外水相产生离子梯度；

3)含药脂质体制备：配制盐酸伊立替康水溶液，加入到具有离子梯度的空白脂质体分散液中，加热搅拌，孵育一定时间即得。

在步骤 3）“含药脂质体制备”步骤后，还可包含以下步骤：

4)游离药物的去除及样品的浓缩：在盐酸伊立替康脂质体中加入缓冲介质，采用切向流装置除去未包封的药物，同时将样品浓缩至适当的体积。

本申请还提供了含有上述脂质体的脂质体注射剂。在将脂质体制备成适合使用的注射剂时，添加稳定剂是有益的。本发明所使用的稳定剂也可选择通常使用的稳定剂，例如维生素 E、乙二胺四乙酸等，这些稳定剂都对制剂的稳定性有所帮助。对于上述处方而言，通过对稳定剂的研究发现乙二胺四乙酸或其盐相对于其他稳定剂效果最好，对于提高脂质体的稳定性最有好处，因此可选用乙二胺四乙酸、乙二胺四乙酸二钠盐、乙二胺四乙酸二钙盐中的一种或多种，且稳定剂的加入比例为 0%～0.5 w/v %且下限不为 0%。

本发明的组合物中优选含有抗氧剂，所述抗氧剂可选自水溶性抗

氧剂或油溶性抗氧剂，所述油溶性抗氧剂选自 α-生育酚、α-生育酸琥珀酸酯、α-醋酸生育酚或其混合物，所述水溶性抗氧剂选自抗坏血酸、亚硫酸氢钠、亚硫酸钠、焦亚硫酸钠、L-半胱氨酸或其混合物，抗氧剂的加入比例为 0%～0.5 w/v %且下限不为 0%。

注射剂可以是注射液或冻干粉针形式。制剂中可含有渗透压调节剂，所述渗透压调节剂选自葡萄糖、蔗糖、山梨醇、甘露醇、氯化钠、甘油、组氨酸及其盐酸化物、甘氨酸及其盐酸化物、赖氨酸、丝氨酸、谷氨酸、精氨酸或缬氨酸中的一种或多种，且渗透压调节剂的加入比例为 0%~5 w/v %且下限不为 0%。

对于冻干粉形式的制剂来说，注射剂进一步含有冻干保护剂，进行冷冻干燥后制得脂质体冻干粉针。冻干保护剂选自葡萄糖、蔗糖、海藻糖、甘露醇、右旋糖酐或乳糖中的一种或多种。

本发明优选的注射剂脂质体含有以下重量配比的成分：

盐酸伊立替康	1 份
氢化大豆卵磷脂	3.4-3.8 份
聚乙二醇 2000-二硬酯酰磷脂酰乙醇胺	0.34-0.38 份
胆固醇	0.8-0.95 份
乙二胺四乙酸二钠	0.05-0.09 份，

且胆固醇与氢化大豆卵磷脂的比例为 1：4。

上述注射剂的制备方法包含下述步骤：

1)通过下述 A 至 D 中任何一种方法制备空白脂质体：

A、根据配方选用中性磷脂、胆固醇溶于无水乙醇或无水乙醇-叔丁醇混合溶剂中，与缓冲剂混合，减压除掉乙醇后得到空白脂质体粗品，之后采用高压均质机或/和挤压设备制备空白脂质体至所需的粒度；

B、根据配方选用中性磷脂、胆固醇溶于氯仿或氯仿-甲醇混合溶剂，旋转蒸发形成脂质膜，加入缓冲剂水化得到空白脂质体粗品，之后采用高压均质机或/和挤压设备制备空白脂质体至所需的粒度；

C、根据配方选用中性磷脂、胆固醇与缓冲剂混合，之后采用高压

均质机或/和挤压设备制备空白脂质体至所需的粒度；

D、根据配方选用中性磷脂、胆固醇溶于无水乙醇或无水乙醇-叔丁醇混合溶剂中，与缓冲剂混合，之后采用高压均质机或/和挤压设备制备空白脂质体至所需的粒度；

2)脂质体膜内外水相离子梯度的产生：置换空白脂质体外水相，使脂质体内水相与外水相产生离子梯度；

3)含药脂质体制备：配制盐酸伊立替康水溶液，加入到具有离子梯度的空白脂质体分散液中，加热搅拌，孵育一定时间即得。

在步骤 3）“含药脂质体制备”步骤后，还可包含以下步骤：

4)游离药物的去除及样品的浓缩：在盐酸伊立替康脂质体中加入缓冲介质，采用切向流装置除去未包封的药物，同时将样品浓缩至适当的体积。

在得到脂质体后，还可通过调整脂质体药物浓度，定容，过滤除菌，灌封于小瓶中，得脂质体注射液；或者向脂质体药物样品中加入冻干保护剂，调整药物浓度，定容，过滤除菌，灌封于瓶中，冷冻干燥，得冻干粉针。

本发明的有益效果：

将盐酸伊立替康制成脂质体制剂，克服现有产品和技术的不足，将药物包裹于脂质体内水相中，提高药物稳定性，使药物在体内以内酯环的状态存在，能够长时间维持活性代谢物 SN-38 在血浆中的浓度，以达到增加制剂疗效，降低药物的毒副作用。

本发明的伊立替康或盐酸伊立替康脂质体制剂通过控制特定的药物、磷脂和胆固醇的比例关系，可达到解决脂质体载药量低的问题，药脂比（w/w）可达 0.25 以上，同时药物包封率可达 90%以上，优选的处方可达 95%以上；本发明通过进一步选择胆固醇和磷脂的用量关系，制备的脂质体粒径较小，提高了脂质体的稳定性。本发明还通过对稳定剂的筛选，优选出加入一定比例的乙二胺四乙酸盐明显提高了脂质体的稳定性；脂质体的粒径在 10nm-220nm 之间，分布均匀；性质稳定，伊立替康或盐酸伊立替康脂质体注射剂影响因素实验结果表明，40℃度条件下放置 10 天，粒径和包封率均无显著变化，各项指标符合要求；伊立替康或盐酸伊立替康脂质体注射剂与市售制剂相比，

抑瘤率显著提高，毒性显著降低。

附图说明

图 1 显示本发明伊立替康或盐酸伊立替康脂质体注射剂的粒径分布。

图 2 显示本发明伊立替康或盐酸伊立替康脂质体注射剂的形态图。

图 3 显示本发明伊立替康或盐酸伊立替康脂质体注射剂的体内抗肿瘤药效试验的结果。

具体实施方式

下面实施例用于进一步说明本发明，但本发明并不仅限于此实施例。

实施例 1

处方

盐酸伊立替康	0.28g	0.28g	0.28g	0.28g	0.28g
氢化大豆卵磷脂	1g	1g	1g	1g	1g
胆固醇	0.4g	0.33g	0.25g	0.2g	0.167g
DSPE-PEG_{2000}	0.1g	0.1g	0.1g	0.1g	0.1g
硫酸铵	5g	5g	5g	5g	5g
氯化钠	0.45g	0.45g	0.45g	0.45g	0.45g
胆固醇：磷脂	1：2.5	1：3	1：4	1：5	1：6
注射用水	加至所需体积				

制备方法：

将处方量的氢化大豆卵磷脂（HSPC）、胆固醇（CHOL）溶于适量无水乙醇得脂质溶液，与 100ml 硫酸铵溶液混合，减压除掉乙醇得到空白脂质体粗品。之后采用高压均质机 1000bar 均质 5 次循环，之后通过挤出设备挤出脂质体控制其粒径（挤出器铺 2 张 0.1μm 挤出膜，挤出 5 次）。之后加入配制好的 DSPE-PEG_{2000} 水溶液，搅拌孵育 20 分钟。采用切向流超滤装置透析空白脂质体，中间不间断的补充注射用水，即得空白脂质体。

用注射用水配制盐酸伊立替康水溶液，按照盐酸伊立替康与 HSPC 的重量比例为 1:3.5 加入到上述具有离子梯度的空白脂质体分散液中，

60℃加热搅拌，孵育 20 分钟即得载药脂质体。采用切向流超滤装置除去未包封的药物，同时将样品浓缩至约 50ml，加入 0.45g 氯化钠调节渗透压。调整药物浓度，定容，0.22 μm 滤膜过滤除菌，充氮气灌封于小瓶中，即得盐酸伊立替康脂质体注射剂。

HSPC：CHOL	制备方法	平均粒径
6：1	均质后	138.7
	0.1μm 挤出 5 次	92.26
5：1	均质后	136.2
	0.1μm 挤出 5 次	89.5
4：1	均质后	123.4
	0.1μm 挤出 5 次	87.26
3：1	均质后	145.1
	0.1μm 挤出 5 次	93.4
2.5：1	均质后	142
	0.1μm 挤出 5 次	98.56

制备的各处方粒径变化如下表所示，结果表明，磷脂与胆固醇重量比为 4:1 时样品粒径最小。

将不同磷脂与胆固醇重量比制备的样品在 25℃条件下放置，考察稳定性。结果如下表，25℃条件下放置 60 天，磷脂与胆固醇重量比为 4:1 样品粒径及包封率无明显变化，其它比例的样品粒径增大，包封率也有所下降，可见，磷脂与胆固醇重量比为 4:1 的样品稳定性较好。

HSPC/CHOL	放置时间（25℃，天）	外观性状	EN%	粒径（z-v）nm	电位（mv）	含量（mg/ml）	总杂质%	溶血磷脂
6：1	0	类白色混悬液	98.86	92.3	-30.5	5.05	0.58	0.39
	30	类白色混悬液	98.56	94.3	-26.8	5.04	0.75	0.56
	60	类白色混悬液	98.20	95.9	-24.9	5.06	0.85	0.66
4：1	0	类白色混悬液	99.37	87.3	-32.1	5.10	0.55	0.40
	30	类白色混悬液	99.25	87.5	-30.9	5.11	0.64	0.50
	60	类白色	99.18	87.8	-28.6	5.09	0.70	0.62

		混悬液						
2.5：1	0	类白色混悬液	99.27	98.5	-35.8	5.12	0.60	0.38
	30	类白色混悬液	98.75	100.2	-28.6	5.09	0.73	0.51
	60	类白色混悬液	98.19	101.7	-25.3	5.07	0.84	0.67

结论：综合各项指标，结果证明胆固醇与磷脂之间的比例控制在 1：3～5 可达到比较好的使用效果，最佳配比为 1：4。

实施例 2

处方

盐酸伊立替康	0.28g
氢化大豆卵磷脂（HSPC）	1g
聚乙二醇 2000-二硬酯酰磷脂酰乙醇胺（$DSPE\text{-}PEG_{2000}$）	0.1g
胆固醇	0.25g
硫酸铵	5g
乙二胺四乙酸二钠	0.02g
氯化钠	0.45g
注射用水	加至所需体积

制备方法：

将处方量的氢化大豆卵磷脂、胆固醇溶于适量无水乙醇得脂质溶液，与 100ml 硫酸铵溶液混合，减压除掉乙醇得到空白脂质体粗品。之后采用高压均质机 1000bar 均质 5 次循环，之后通过挤出设备挤出脂质体控制其粒径（挤出器铺 2 张 0.1μm 挤出膜，挤出 5 次）。之后加入配制好的 $DSPE\text{-}PEG_{2000}$ 水溶液，搅拌孵育 20 分钟。采用切向流超滤装置透析空白脂质体，中间不间断的补充注射用水，即得空白脂质体。

用注射用水配制盐酸伊立替康水溶液，按照盐酸伊立替康与 HSPC 的重量比例为 1:3.5 加入到上述具有离子梯度的空白脂质体分散液中，60℃加热搅拌，孵育 20 分钟即得载药脂质体。采用切向流超滤装置除去未包封的药物，同时将样品浓缩至约 50ml，加入 0.45g 氯化钠调节

渗透压。调整药物浓度，定容，0.22 μm 滤膜过滤除菌，充氮气灌封于小瓶中，即得盐酸伊立替康脂质体注射剂。

实施例 3

空白脂质体处方及制备方法与实施例 2 相同，不同的是按照盐酸伊立替康与 HSPC 的重量比例分别为为 1:1.5、1:2、1:3.5、1:4 及 1:5 进行制备脂质体，盐酸伊立替康脂质体样品包封率及粒径见下表。

CPT11/HSPC	包封率（%）	载药量（mg/ml）	粒径（nm）
1:1.5	83.2	5.11	87.1
1:2	90.8	5.15	86.5
1:3.5	99.4	5.08	85.9
1:4	99.1	4.81	85.4
1:5	99.4	4.25	86.7

结果说明，当盐酸伊立替康与 HSPC 的比例为 1:1.5 时包封率显著降低，而当其比例为 1:5 时载药量下降明显，不适合制备成临床应用的制剂，当其比例在 1:2 与 1:4 之间时包封率及载药量均较高。

实施例 4

按照实施例 2 中处方组份的用量来制备空白脂质体及载药脂质体，制备方法同实施例 2 所述方法，所不同的是分别采用高纯蛋黄磷脂（EPC）、高纯大豆磷脂（SPC）替换处方中的 HSPC 来制备。将制备的脂质体样品置于 25℃条件下考察稳定性。结果见下表。试验结果表明，采用 HSPC 制备的脂质体样品稳定性最好，25℃条件下放置 2 个月主要指标无明显变化。

时间	PC 组成	包封率（%）	载药量（mg/ml）	粒径（nm）
0M	HSPC	99.4	5.08	85.9
	EPC	99.5	5.10	87.5
	SPC	99.2	5.01	86.9
1M	HSPC	99.5	5.10	85.5
	EPC	92.4	5.07	88.2
	SPC	93.9	5.05	87.3

	HSPC	98.7	5.07	86.5
2M	EPC	85.8	5.06	93.2
	SPC	89.6	5.02	91.5

实施例 5

处方

盐酸伊立替康	0.28g
氢化大豆卵磷脂（HSPC）	1g
聚乙二醇 2000-二硬酯酰磷脂酰乙醇胺（$DSPE\text{-}PEG_{2000}$）	0.1g
胆固醇	0.25g
生理盐水溶液	50ml
注射用水	加至所需体积

制备方法 <1> ：

乙醇注入法：将处方量的氢化大豆卵磷脂、$DSPE\text{-}PEG_{2000}$、胆固醇溶于适量无水乙醇得脂质溶液，注入到盐酸伊立替康的生理盐水溶液中，减压除掉乙醇得到脂质体粗品。之后采用高压均质机 1000bar 均质 5 次循环，之后通过挤出设备挤出脂质体控制其粒径（挤出器铺 2 张 0.1μm 挤出膜，挤出 5 次）。调整药物浓度，定容，0.22 μm 滤膜过滤除菌，充氮气灌封于小瓶中，即得盐酸伊立替康脂质体注射剂。

制备方法 <2>：

薄膜分散法：将处方量的氢化大豆卵磷脂、$DSPE\text{-}PEG_{2000}$、胆固醇溶于适量氯仿得脂质溶液，将脂质溶液旋转蒸发成膜，除尽氯仿然后加入盐酸伊立替康的生理盐水溶液水化孵育约 1h。之后采用高压均质机 1000bar 均质 5 次循环，再通过挤出设备挤出脂质体控制其粒径（挤出器铺 2 张 0.1μm 挤出膜，挤出 5 次）。调整药物浓度，定容，0.22 μm 滤膜过滤除菌，充氮气灌封于小瓶中，即得盐酸伊立替康脂质体注射剂。

测定制备方法<1>、<2>以及实施例 2 制备的盐酸伊立替康脂质体包封率及粒径。

样品	包封率（%）	粒径（nm）

实施例 2	99.4	85.9
制备方法<1>	15.3	87.9
制备方法<2>	17.8	90.2

结果表明，采用被动载药法如乙醇注入法及薄膜蒸发法制备盐酸伊立替康脂质体时，可以制备出目的产品。但其样品包封率较低，大部分药物都没有进入到脂质体中；而采用主动载药法制备的样品（实施例 2）包封率高，载药量高，粒径小而均匀，所以采用主动载药法。本发明中即采用离子梯度法制备盐酸伊立替康脂质体有非常好的效果。

实施例 6

处方	处方 1	处方 2	处方 3	处方 4
HSPC	1 g	1 g	1 g	1 g
胆固醇	250 mg	250 mg	250 mg	250 mg
PEG_{2000}-DSPE	0.1 g	0.1 g	0.1 g	0.1 g
维生素 E	/	0.02 g	/	0.02 g
EDTA-2Na	/	/	0.02 g	0.02 g
硫酸铵溶液（300mM）	100 ml	100 ml	100 ml	100 ml
盐酸伊立替康	0.3 g	0.3 g	0.3 g	0.3 g

制备方法：

空白脂质体：将脂质乙醇注入。均质 1000bar，6 次；200nm 挤出 3 次，100 nm 挤出 5 次；加入 PEG_{2000}-DSPE，60℃赋予 30 min。切向流透析 3 次，每次加入 50 ml。其中 VE 加在磷脂有机溶剂中，EDTA 加在硫酸铵溶液中。

载药脂质体：配制约 10mg/ml 的盐酸伊立替康水溶液，加入空白脂质体中，60℃赋予 15 min。用切向流浓缩样品至约 50ml，即为 5mg/ml 样品。

稳定性结果见下表，可见，单独加入乙二胺四乙酸二钠样品各项指标无显著变化，能显著提高了脂质体的稳定性，而其它稳定剂对脂质体的稳定性没有明显改善。

样品名称	放置时间(25℃，天)	外观性状	EN%	粒径(z-v) nm	含量 mg/ml	总杂质%	溶血磷脂（mg/ml）
HSPC	0	类白色混悬液	99.70	85.6	5.42	0.65	0.40
	30	类白色混悬液	91.51	87.7	5.40	0.74	0.65
HSPC+VE	0	类白色混悬液	97.10	89.0	5.01	0.48	0.35
	30	类白色混悬液	93.49	93.4	5.03	0.56	0.43
HSPC+EDTA	0	类白色混悬液	95.67	87.2	4.94	0.56	0.38
	30	类白色混悬液	95.67	86.5	4.98	0.60	0.40
HSPC+VE+EDTA	0	类白色混悬液	98.92	89.2	5.55	0.61	0.39
	30	有颗粒析出	87.31	99.7	5.51	0.61	0.47

实施例 7

处方（1）

盐酸伊立替康	0.5g
氢化大豆卵磷脂	1.5g
胆固醇	0.4g
硫酸锰	10g
甘露醇	2.5g
注射用水	加至所需体积

制备方法：

将处方量的氢化大豆卵磷脂、胆固醇溶于适量无水乙醇得脂质溶液，与 100ml 硫酸锰溶液混合，减压除掉乙醇得到空白脂质体粗品。之后通过挤出设备挤出脂质体控制其粒径（挤出器铺 2 张 0.1μm 挤出膜，挤出 5 次）。采用切向流超滤装置透析空白脂质体，中间不间断的补充注射用水，即得空白脂质体。用注射用水配制盐酸伊立替康水溶液，加入到具有离子梯度的空白脂质体分散液中，50℃加热搅拌，孵育 20 分钟即得载药脂质体。采用切向流超滤装置除去未包封的药物，同时将样品浓缩至约 50ml，加入 2.5g 甘露醇调节渗透压。调整药物浓度，定容，0.22 μm 滤膜过滤除菌，充氮气灌封于小瓶中，即得盐酸伊立替康脂质体注射剂。经过纳米粒度测定仪测得脂质体粒度为 89.3nm，包封率为 97.5%。

处方（2）

盐酸伊立替康	1g
氢化蛋黄卵磷脂（HEPC）	3.45g
胆固醇	0.8g
硫酸镁	10g
组氨酸	2.5g
注射用水	加至所需体积

制备方法：

将处方量的氢化蛋黄卵磷脂、胆固醇溶于适量无水乙醇得脂质溶液，与 100ml 硫酸镁溶液混合。之后通过挤出设备挤出脂质体控制其粒径（挤出器铺 2 张 0.1μm 挤出膜，挤出 5 次）。采用切向流超滤装置透析空白脂质体，中间不间断的补充注射用水，即得空白脂质体。用注射用水配制盐酸伊立替康水溶液，加入到具有离子梯度的空白脂质体分散液中，50℃加热搅拌，孵育 20 分钟即得载药脂质体。采用切向流超滤装置除去未包封的药物，同时将样品浓缩至约 50ml，加入 2.5g 组氨酸调节渗透压。调整药物浓度，定容，0.22 μm 滤膜过滤除菌，充氮气灌封于小瓶中，即得盐酸伊立替康脂质体注射剂。经过纳米粒度测定仪测得脂质体粒度为 87.6nm，包封率为 98.1%。

处方（3）

盐酸伊立替康	0.3g
氢化大豆卵磷脂（HSPC）	1g
聚乙二醇 2000-二硬酯酰磷脂酰乙醇胺（$DSPE\text{-}PEG_{2000}$）	0.05g
胆固醇	0.25g
硫酸铵	5g
氯化钠	0.45g
注射用水	加至所需体积

制备方法：

将处方量的氢化大豆卵磷脂、胆固醇溶于适量无水乙醇得脂质溶液，与 100ml 硫酸铵溶液混合，减压除掉乙醇得到空白脂质体粗品。之后采用高压均质机 1000bar 均质 5 次循环后，之后加入配制好的 $DSPE\text{-}PEG_{2000}$ 水溶液，搅拌孵育 20 分钟。采用切向流超滤装置透析空

白脂质体，中间不间断的补充注射用水，即得空白脂质体。用注射用水配制盐酸伊立替康水溶液，加入到具有离子梯度的空白脂质体分散液中，60℃加热搅拌，孵育 20 分钟即得载药脂质体。采用切向流超滤装置除去未包封的药物，同时将样品浓缩至约 50ml，加入 0.45g 氯化钠调节渗透压。调整药物浓度，定容，0.22 μm 滤膜过滤除菌，充氮气灌封于小瓶中，即得盐酸伊立替康脂质体注射剂。经过纳米粒度测定仪测得脂质体粒度为 87.3nm，包封率为 99.2%。

实施例 8

处方

盐酸伊立替康	0.5g
氢化大豆卵磷脂（HSPC）	1g
心肌磷脂（CL）	0.5g
聚乙二醇 5000-二硬酯酰磷脂酰乙醇胺（DSPE-PEG_{5000}）	0.5g
α-生育酚	0.05g
胆固醇	0.35g
柠檬酸	5.76g
氯化钠	约 3.6g
注射用水	加至所需体积

制备方法：

将处方量的氢化大豆卵磷脂、心肌磷脂、DSPE-PEG_{5000}、胆固醇、α-生育酚溶于适量无水乙醇得脂质溶液，与 100ml 柠檬酸溶液混合，减压除掉乙醇得到空白脂质体粗品。之后采用高压均质机 1000bar，均质 5 次。采用切向流超滤装置透析空白脂质体，中间不间断的补充 0.9% 氯化钠溶液 400ml，即得空白脂质体。用注射用水配制盐酸伊立替康溶液，加入到具有离子梯度的空白脂质体分散液中，60℃加热搅拌，孵育 20 分钟即得载药脂质体。采用切向流超滤装置除去未包封的药物，同时将样品浓缩至约 50ml。调整药物浓度，定容，0.22 μm 滤膜过滤除菌，充氮气灌封于小瓶中，即得盐酸伊立替康脂质体注射剂。经过纳米粒度测定仪测得脂质体粒度为 85.8nm，包封率为 98.6%。

实施例 9

处方

盐酸伊立替康	0.8g
二棕榈酰磷脂酰胆碱(DPPC)	2g
二棕榈酰磷脂酰甘油(DPPG)	0.2g
胆固醇	0.5g
抗坏血酸	0.05g
乙二胺四乙酸二钠	0.05g
硫酸铵	5g
氯化钠	约 3.6g
注射用水	加至所需体积

制备方法：

将处方量的 DPPC、DPPG、胆固醇溶于适量无水乙醇得脂质溶液，与 100ml 硫酸铵溶液（含乙二胺四乙酸二钠）混合，减压除掉乙醇得到空白脂质体粗品。之后采用高压均质机 1000bar，均质 5 次。采用切向流超滤装置透析空白脂质体，中间不间断的补充 0.9%氯化钠溶液 400ml，即得空白脂质体。用注射用水配制盐酸伊立替康溶液，加入到具有离子梯度的空白脂质体分散液中，60℃加热搅拌，孵育 20 分钟即得载药脂质体。采用切向流超滤装置除去未包封的药物，同时将样品浓缩至约 50ml，加入抗坏血酸。调整药物浓度，定容，0.22 μm 滤膜过滤除菌，充氮气灌封于小瓶中，即得盐酸伊立替康脂质体注射剂。经过纳米粒度测定仪测得脂质体粒度为 89.4nm，包封率为 97.2%。

实施例 10

处方

盐酸伊立替康	0.5g
氢化大豆卵磷脂（HSPC）	1g
聚乙二醇 5000-二硬酯酰磷脂酰乙醇胺（DSPE-PEG_{2000}）	0.1g
α-生育酚	0.05g
胆固醇	0.3g
硫酸铵	5g
氯化钠	约 3.6g
蔗糖	2g

甘露醇	1g
注射用水	加至所需体积

制备方法：

将处方量的氢化大豆卵磷脂、胆固醇、α-生育酚溶于适量无水乙醇得脂质溶液，与 100ml 硫酸铵溶液混合，减压除掉乙醇得到空白脂质体粗品。之后采用高压均质机 1000bar，均质 5 次，再通过挤出设备挤出脂质体（挤出器铺 5 张 100nm 挤出膜，挤出 5 次），之后加入配制好的 DSPE-PEG_{5000} 水溶液，搅拌孵育 20 分钟。采用切向流超滤装置透析空白脂质体，中间不间断的补充 0.9%氯化钠溶液 400ml，即得空白脂质体。用注射用水配制盐酸伊立替康溶液，加入到具有离子梯度的空白脂质体分散液中，60℃加热搅拌，孵育 20 分钟即得载药脂质体。采用切向流超滤装置除去未包封的药物，同时将样品浓缩至约 50ml，加入蔗糖和甘露醇使混合均匀。调整药物浓度，定容，0.22 μm 滤膜过滤除菌，罐装于西林瓶中，冷冻干燥，即得盐酸伊立替康脂质体冻干粉针。将脂质体冻干粉针水化后测定脂质体粒度为 90.8nm，包封率为 97.5%。

试验例 1

根据本发明制得的成品（按实施例 2）的理化特征：

【粒径分布】取本品适量，以水稀释后用动态光散射法进行测定。测定波长 λ=633nm，测定角度 173°，温度 25℃。粒径大小以强度径（Intensity）数据表示。粒径分布图见图 1，平均粒径为 85.9nm。

【形态学考察】 吸取脂质体样品适量，将铜网置于干净滤纸上，向铜网上滴加样品，用 2%磷钨酸染色，待干燥后在 JEM2010 透射电镜（日本电子株式会社）下观察本品。形态图见图 2。经透射电镜观察，伊立替康脂质体形态均为典型的双分子层结构，粒径大部分在 200 nm 以下，与动态光散射法测定的结果相吻合。

【包封率测定】 药物含量测定方法：色谱柱：Agilent ZORBAX Eclipse XDB-C18（4.6×150mm,5μm）；流动相：乙腈- 0.05M 磷酸二氢钾缓冲液（pH 4，含 1%三乙胺）= 20:80；柱温：40℃ ；进样体积：20μL；流速：1.0mL/min。

包封率测定方法：吸取 1mL 样品溶液至 10mL 量瓶中，加入水稀释至刻度，摇匀，置 8010 型超滤器（MILLIPORE 公司）超滤，弃去初滤液，取续滤液为供试品溶液。分别吸取供试品溶液、对照品溶液 20μL 注入液相色谱仪，记录色谱图，以外标法计算制剂游离药物含量，记为 W；另按含量测定项下方法计算本品的药物总含量，记为 W_0。按下式计算样品的包封率。

$$包封率 = \frac{W_0 - W}{W_0} \times 100\%$$

测定结果：本品包封率为 99.4。

【影响因素试验】 取本品置于不同条件下进行影响因素考察，结果如下表所示：

试验条件	放置时间（天）	外观性状	pH	粒径(nm)	含量(%)	包封率(%)	总杂质（%）	溶血磷脂（mg/ml）
光照 4500lx±500lx	0	类白色混悬液	6.39	85.9	98.14	99.40	0.43	0.19
	5	土黄色混悬液	6.30	86.3	78.99	99.11	14.4	0.23
	10	土黄色混悬液	6.40	86.5	76.39	99.20	19.5	0.30
40℃	0	类白色混悬液	6.39	85.9	98.14	99.40	0.43	0.19
	5	类白色混悬液	6.35	87.1	98.77	99.29	0.45	0.29
	10	类白色混悬液	6.47	88.7	98.86	96.82	0.55	0.44
低温	三个循环	类白色混悬液	6.41	89.1	100.07	99.16	0.44	0.38
冻融	三个循环	白色混悬液	6.38	110.5	95.22	99.28	0.46	0.23

结果表明，样品对光照敏感，经过强光照射样品外观变黄，含量下降，有关物质明显增大；样品在高温 40°C 时包封率和粒径无明显变化，但有关物质略增大；低温和冷冻循环试验表明样品产生大粒子。综合考虑磷脂在高温状态下的不稳定性，结合影响因素试验结果，本品应低温避光贮存。

【体内抗肿瘤药效试验】

药物名称：按实施例 2 制备的盐酸伊立替康脂质体（CPT-11 脂质体）由上海恒瑞医药有限公司提供，盐酸伊立替康注射液（CPT-11，江苏恒瑞医药股份有限公司提供）

配制方法：均用生理盐水配成所需浓度。

实验动物：BALB/cA-nude 裸小鼠，6-7 周，♀，购自上海斯莱克实验动物有限责任公司。合格证号：SCXK（沪）2007－0005。饲养环境：SPF 级。

试验方法：裸小鼠皮下接种人结肠癌 Ls-174t 细胞，待肿瘤生长至 150-300mm^3 后，将动物随机分组(d0)。给药剂量和给药方案见下表。每周测 2－3 次瘤体积，称鼠重，记录数据。肿瘤体积（V）计算公式为：

$V=1/2\times a\times b^2$ 其中 a、b 分别表示长、宽。

药物	给药	途径	平均肿瘤体积(mm3) D0	SD	平均肿瘤体积(mm3) D12	SD	RTV D12	SD	抑瘤率 % T/C D12	抑瘤率 (%) D12	p 值 D12	部分消退	动物数
Vehicle	D0、3	IV	219.8	± 37.2	2013.7	± 303.1	9.4	± 2.3	100	0	-	0	8
CPT-11脂质体 1.0mg/kg	D0、3	IV	212.2	± 42.1	732.2	± 162.6	3.5	± 0.7	38	62	0.000	0	13
CPT-11脂质体 3.0mg/kg	D0、3	IV	205.0	± 49.0	265.1	± 122.9	1.3	± 0.4	13	87	0.000	4	13
CPT-11 10mg/kg	D0、3	IV	204.6	± 44.7	844.4	± 197.5	4.2	± 0.9	45	55	0.000	0	14

d0: 第一次给药时间；RTV：相对肿瘤体积；P value 指与对照相比。对照组 n=12，治疗组 n=6。

实验结果：

CPT-11 脂质体及 CPT-11 均明显抑制人结肠癌 Ls-174t 裸小鼠移植瘤的生长，CPT-11 脂质体抑制 Ls-174t 生长有明显的剂量依赖性，高剂量（3mg/kg）给药时，有 4/14 个肿瘤部分消退，低剂量（1mg/kg）给药时的疗效与 CPT-11 10mg/kg 给药时相当，提示 CPT-11 脂质体的疗效有可能比其注射液至少提高 10 倍，具体结果见图 3。

权利要求书：

1、一种伊立替康或盐酸伊立替康脂质体，其特征在于所述脂质体含有伊立替康或盐酸伊立替康、中性磷脂以及胆固醇，其中所述胆固醇与中性磷脂的重量比例为 1：3~5。

2、根据权利要求 1 所述的脂质体，其特征在于所述中性磷脂和伊立替康或盐酸伊立替康符合以下重量配比：

伊立替康或盐酸伊立替康	1 份
中性磷脂	2～5 份，优选 2.5-4 份。

3、根据权利要求 1 所述的脂质体，其特征在于所述中性磷脂含有氢化大豆卵磷脂。

4、根据权利要求 3 所述的脂质体，其特征在于所述中性磷脂是氢化大豆卵磷脂。

5、根据前述任意一项权利要求所述的脂质体，其特征在于所述胆固醇与中性磷脂的比例为 1：3.5~4.5，最优选为 1：4。

6、根据前述任意一项权利要求所述的脂质体，其特征在于在所述脂质体通过离子梯度法制备得到。

7、根据权利要求 6 所述的脂质体，其特征在于在所述脂质体内水相与外水相之间具有缓冲剂形成的离子梯度，优选所述脂质体内水相比外水相具有离子浓度高的离子梯度。

8、根据前述任意一项权利要求所述的脂质体，其特征在于所述脂质体还含有亲水性高分子的脂质衍生物，优选是 DSPE-PEG_{2000}。

9、根据权利要求 8 所述的脂质体，其特征在于所述亲水性高分子的脂质衍生物与伊立替康或盐酸伊立替康的重量比为 0.2～0.4。

10、根据前述任意一项权利要求所述的脂质体，其特征在于所述脂质体进一步含有带电荷磷脂，所述带电荷磷脂选自二月桂酰磷脂酰甘油、二棕榈酰磷脂酰甘油、二硬脂酰磷脂酰甘油、二肉豆蔻酰磷脂酰甘油、二油酸磷脂酰丝氨酸、二油酰磷脂酰甘油、二月桂酰磷脂酸、二肉豆蔻酰磷脂酸或二硬脂酰磷脂酸中的一种或多种，且带电荷磷脂与中性磷脂的重量比例为 1:5～1:100。

11、根据权利要求 1 所述的脂质体，其特征在于所述脂质体含有以下重量配比的成分：

盐酸伊立替康	1 份
氢化大豆卵磷脂	3.4-3.8 份
聚乙二醇 2000-二硬酯酰磷脂酰乙醇胺	0.34-0.38 份
胆固醇	0.8-0.95 份，

且胆固醇与氢化大豆卵磷脂的比例为 1：4。

12、权利要求 1-11 任意一项所述脂质体的制备方法，其特征在于该制备方法包含下述步骤：

1)通过下述 A 至 D 中任何一种方法制备空白脂质体：

A.根据配方选用中性磷脂、胆固醇溶于无水乙醇或无水乙醇-叔丁醇混合溶剂中，与缓冲剂混合，减压除掉乙醇后得到空白脂质体粗品，之后采用高压均质机或/和挤压设备制备空白脂质体至所需的粒度；

B.根据配方选用中性磷脂、胆固醇溶于氯仿或氯仿-甲醇混合溶剂，旋转蒸发形成脂质膜，加入缓冲剂水化得到空白脂质体粗品，之后采用高压均质机或/和挤压设备制备空白脂质体至所需的粒度；

C.根据配方选用中性磷脂、胆固醇与缓冲剂混合，之后采用高压均质机或/和挤压设备制备空白脂质体至所需的粒度；

D.根据配方选用中性磷脂、胆固醇溶于无水乙醇或无水乙醇-叔丁醇混合溶剂中，与缓冲剂混合，之后采用高压均质机或/和挤压设备制备空白脂质体至所需的粒度；

2)脂质体膜内外水相离子梯度的产生：置换空白脂质体外水相，使脂质体内水相与外水相产生离子梯度；

3)含药脂质体制备：配制盐酸伊立替康水溶液，加入到具有离子梯度的空白脂质体分散液中，加热搅拌，孵育，即得。

13、根据权利要求 12 所述的制备方法，其特征在于在 3）含药脂质体制备步骤后，还包含以下步骤：

4)游离药物的去除及样品的浓缩：在盐酸伊立替康脂质体中加入缓冲介质，采用切向流装置除去未包封的药物，同时将样品浓缩至适当的体积。

14、根据权利要求 12-13 任意一项所述的制备方法，其特征在于所述缓冲剂选自含有 Na^+、K^+、Fe^{2+}、Ca^{2+}、Ba^{2+}、Mn^{2+}、Mg^{2+}、Li^+、NH_4^+ 或 H^+离子盐的一种或多种。

15、含有权利要求 1-11 任意一项所述伊立替康或盐酸伊立替康脂质体的脂质体注射剂。

16、根据权利要求 15 所述的脂质体注射剂，其特征在于所述注射剂含有稳定剂，所述稳定剂选自乙二胺四乙酸、乙二胺四乙酸二钠盐、乙二胺四乙酸二钙盐中的一种或多种，稳定剂的加入比例为 0%～0.5 w/v %且下限不为 0%，优选是乙二胺四乙酸二钠。

17、根据权利要求 15 所述的脂质体注射剂，其特征在于所述注射剂是注射液或冻干粉针。

18、根据权利要求 15 所述的脂质体注射剂，其特征在于所述注射剂含有渗透压调节剂，所述渗透压调节剂选自葡萄糖、蔗糖、山梨醇、甘露醇、氯化钠、甘油、组氨酸及其盐酸化物、甘氨酸及其盐酸化物、赖氨酸、丝氨酸、谷氨酸、精氨酸或缬氨酸中的一种或多种，渗透压调节剂的加入比例为 0%~5 w/v %且下限不为 0%。

19、根据权利要求 15 所述的脂质体注射剂，其特征在于所述注射剂进一步含有抗氧剂，所述抗氧剂选自水溶性抗氧剂或油溶性抗氧剂，

所述油溶性抗氧剂选自 α-生育酚、α-生育酸琥珀酸酯、α-醋酸生育酚或其混合物，所述水溶性抗氧剂选自抗坏血酸、亚硫酸氢钠、亚硫酸钠、焦亚硫酸钠、L-半胱氨酸或其混合物，抗氧剂的加入比例为 0%～0.5 w/v %且下限不为 0%。

20、根据权利要求 17 所述的脂质体注射剂，其特征在于所述注射剂是冻干粉针，其中含有冻干保护剂，是通过冷冻干燥制得的脂质体冻干粉针。

21、根据权利要求 15 所述的脂质体注射剂，其特征在于所述注射剂脂质体含有以下重量配比的成分：：

盐酸伊立替康	1 份
氢化大豆卵磷脂	3.4-3.8 份
聚乙二醇 2000-二硬酯酰磷脂酰乙醇胺	0.34-0.38 份
胆固醇	0.8-0.95 份
乙二胺四乙酸二钠	0.05-0.09 份，

且胆固醇与氢化大豆卵磷脂的比例为 1：4。

22、权利要求 15-21 任意一项所述脂质体注射剂的制备工艺，其特征在于所述工艺包括权利要求 12-13 任意一项的制备方法。

23、根据权利要求 22 的制备工艺，其特征在于所述工艺还包括：

定容、除菌、分装：调整脂质体药物浓度，定容，过滤除菌，灌封于小瓶中，得脂质体注射液；或者

向脂质体药物样品中加入冻干保护剂，调整药物浓度，定容，过滤除菌，灌封于瓶中，冷冻干燥，得冻干粉针。

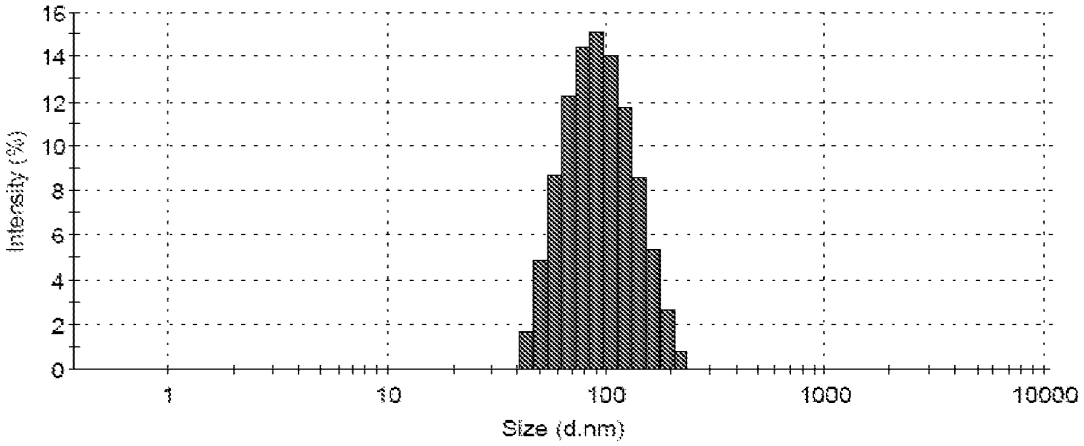

图 1

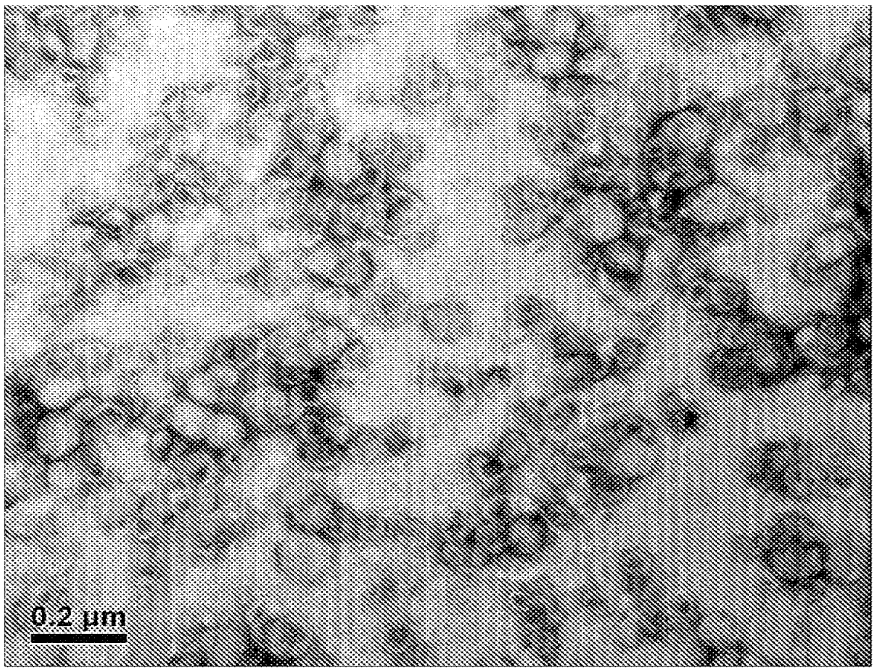

图 2

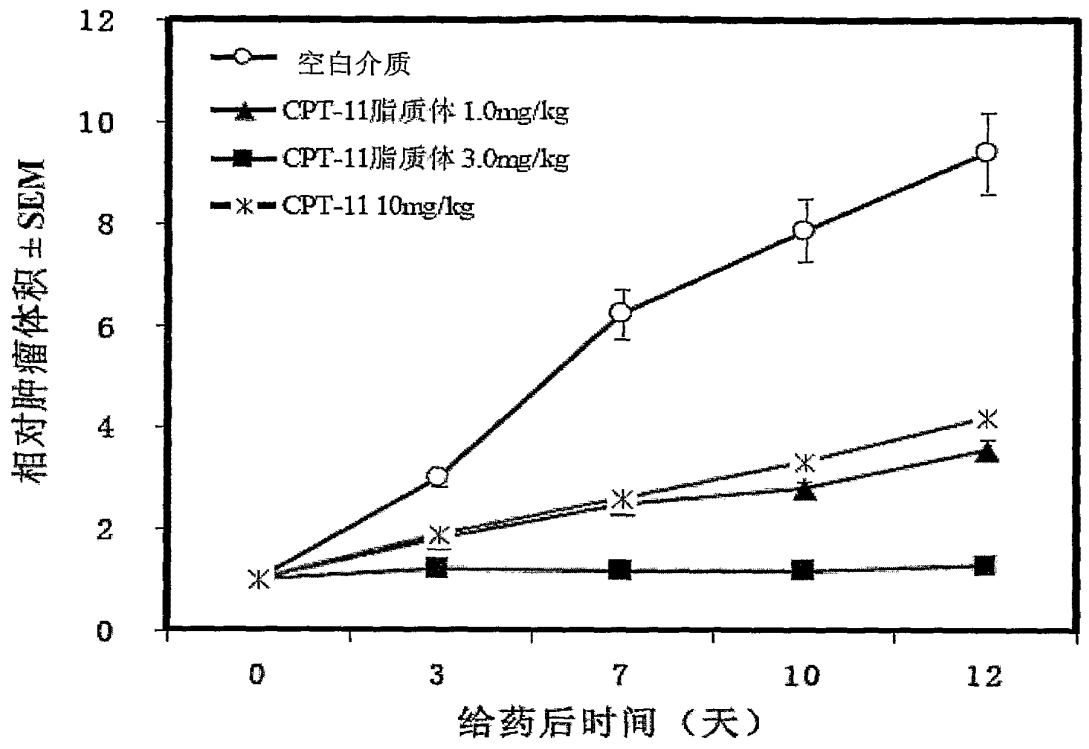
12
10
8
6
4
2
0
相对肿瘤体积±SEM
空白介质
CPT-11脂质体 1.0mg/kg
CPT-11脂质体 3.0mg/kg
CPT-11 10mg/kg
0
3
7
10
12
给药后时间（天）

图 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2009/075298

A. CLASSIFICATION OF SUBJECT MATTER

SEE THE EXTRA SHEET

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:A61K, A61P

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)

DATABASE: WPI, EPODOC, CNPAT, CNKI, CA, EMBASE

KEY WORDS: Camptothecin, topotecan, irinotecan, tpt, hept, hydroxycamptothecin, liposome, Hydrogenated Soya phosphatidyl choline, phosphatidyl, phospholipid, HSPC, SPC, cholesterin, cholesterol, chol,lipid, lipide, liposome

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	QIU Yong-hong, etc., "Preparation and quality evaluation of irinotecan hydrochloride lyophilized liposomes", Journal of Jiangsu University(Medicine Edition),	1-9, 11,12,14-23
Y	Jul. 2009, Vol. 19, No. 4, see pages 315-317, ISSN 1671-7783	10, 13
X	CN1994279A(XIAN LIBANG MEDICINE SCI & TECHNOLOGY CO.),	1-9, 11,12,14-23
Y	11 Jul. 2007(11.07.2007), see example 5, page 2 lines 5-14	10, 13

☒ 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

"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
13 Aug. 2010 (13.08.2010)	**23 Sep. 2010 (23.09.2010)**
Name and mailing address of the ISA/CN The State Intellectual Property Office, the P.R.China 6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China 100088 Facsimile No. 86-10-62019451	Authorized officer LI,Fengyun Telephone No. (86-10)62411143

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2009/075298

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	CN1960729A(TERUMO CORP, et al.), 09. May 2007(09.05.2007), see example 8, page 9 lines 18-22, page 11 lines 9-16	1-9, 11,12,14-23 10, 13
X Y	CN101019834A(XIAN LIBANG MEDICINE SCI & TECHNOLOGY CO.), 22 Aug. 2007(22.08.2007), see example 3	1-9, 11,12,14-23 10, 13
X Y A	CN101283983A(NANJING CHANGAO MEDICINE SCI TECH CO. LT.), 15 Oct. 2008(15.10.2008), see example 3	1-9, 11,15-21 10 12-14,22,23
X Y A	CN1650864A(UNIV FUDAN), 10 Aug. 2005(10.08.2005), see page 4 lines 26-29, example 1	1-9, 11,15-21 10 12-14,22,23
X Y	YANG Jian-kun, etc. , Study on preparation of irinotecan hydrochloride liposomes and Influence factors, Chinese Journal of New Drugs, 2007, Vol. 16, No. 23, see pages	1-9, 11,12,14-23 10, 13
A	WO200811427A1(DABUR PHARMA LIMITED), 25 Sep. 2008(25.09.2008) see example 8	1-23
A	CN1323199A(ALZA CORP), 21 Nov. 2001(21.11.2001) see example 1	1-23

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2009/075298

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
CN1994279A	11.07.2007	NONE	
CN1960729A	09.05.2007	WO2005117878A1	15.12.2005
		EP1752150A1	14.02.2007
		NO20066074A	28.02.2007
		AU2005249314A1	15.12.2005
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		KR20070037440A	04.04.2007
		BRPI0511753A	02.01.2008
		ZA200610204A	30.01.2008
		US2008069868A1	20.03.2008
		JP2006514098T2	03.04.2008
		MXPA06013874A	01.05.2007
		AU2005249314B2	11.09.2008
		KR100889139B1	17.03.2009
		TW200600095A	01.01.2006
		IN225498B	13.02.2009
		CA2567857C	22.12.2009
		NZ551748A	26.02.2010
CN101019834A	22.08.2007	NONE	
CN1650864A	10.08.2005	CN1326525C	18.07.2007
CN101283983A	15.10.2008	NONE	
WO200811427A1	25.09.2008	US2009017105A1	15.01.2009
		INDEL200700590A	13.02.2009
		AU2008227852A1	25.09.2008
		CA2681302A1	25.09.2008

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2009/075298

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
		EP2146692A1	27.01.2010
CN1323199A	21.11.2001	WO0023052A1	27.04.2000
		AU1118900A	08.05.2000
		NO20011844A	10.04.2001
		EP1121102A1	08.08.2001
		BR9914601A	23.10.2001
		KR20010075639A	09.08.2001
		ZA200103063A	24.12.2001
		US6355268B1	12.03.2002
		US2002146450A1	10.10.2002
		JP2002527466T	27.08.2002
		US6465008B1	15.10.2002
		EP1121102B1	23.04.2003
		DE69907243E	28.05.2003
		US2003133973A1	17.07.2003
		MXPA01003796A	01.03.2002
		NZ511112A	28.11.2003
		ES2198970T3	01.02.2004
		AU774715B2	08.07.2004
		MX220153B	28.04.2004
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		CN1205923C	15.06.2005
		US7244449B2	17.07.2007
		US2007231379A1	04.10.2007
		US7279179B2	09.10.2007
		KR100679906B1	07.02.2007

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2009/075298

International Patent Classification (IPC)

A61K9/127(2006.01)i
A61K31/4745(2006.01)i
A61P35/00(2006.01)i

国际检索报告

国际申请号
PCT/CN2009/075298

A. 主题的分类

参见附加页

按照国际专利分类(IPC)或者同时按照国家分类和 IPC 两种分类

B. 检索领域

检索的最低限度文献(标明分类系统和分类号)

IPC:A61K, A61P

包含在检索领域中的除最低限度文献以外的检索文献

在国际检索时查阅的电子数据库(数据库的名称，和使用的检索词（如使用）)

数据库：WPI, EPODOC, CNPAT, CNKI, CA, EMBASE

检索词：喜树碱，羟基喜树碱，拓扑替康，伊立替康，托泊替康，勒托替康，磷脂，氢化大豆卵磷脂，胆固醇，脂质，脂质体，Camptothecin, topotecan, irinotecan, tpt, hcpt, hydroxycamptothecin, liposome, Hydrogenated Soya phosphatidyl choline, phosphatidyl, phospholipid, HSPC, SPC, cholesterin, cholesterol, chol,lipid, lipide, liposome

C. 相关文件

类　型*	引用文件，必要时，指明相关段落	相关的权利要求
X	邱永宏 等，盐酸伊立替康脂质体冻干粉针的制备及其质量评价，江苏大学学报（医学版），2009 年 7 月，第 19 卷第 4 期，第 315－317 页 ISSN 1671-7783	1-9, 11,12,14-23
Y		10, 13
X	CN1994279A(西安力邦医药科技有限责任公司)，11.7 月 2007（11.07.2007）	1-9, 11,12,14-23
Y	参见实施例 5，说明书第 2 页第 5－14 行	10, 13

☒ 其余文件在 C 栏的续页中列出。　　☒ 见同族专利附件。

* 引用文件的具体类型：

"A" 认为不特别相关的表示了现有技术一般状态的文件

"E" 在国际申请日的当天或之后公布的在先申请或专利

"L" 可能对优先权要求构成怀疑的文件，或为确定另一篇引用文件的公布日而引用的或者因其他特殊理由而引用的文件（如具体说明的）

"O" 涉及口头公开、使用、展览或其他方式公开的文件

"P" 公布日先于国际申请日但迟于所要求的优先权日的文件

"T" 在申请日或优先权日之后公布，与申请不相抵触，但为了理解发明之理论或原理的在后文件

"X" 特别相关的文件，单独考虑该文件，认定要求保护的发明不是新颖的或不具有创造性

"Y" 特别相关的文件，当该文件与另一篇或者多篇该类文件结合并且这种结合对于本领域技术人员为显而易见时，要求保护的发明不具有创造性

"&" 同族专利的文件

国际检索实际完成的日期	国际检索报告邮寄日期
13.8 月 2010（13.08.2010）	**23.9 月 2010 (23.09.2010)**
ISA/CN 的名称和邮寄地址： 中华人民共和国国家知识产权局 中国北京市海淀区蓟门桥西土城路 6 号 100088 传真号：　(86-10)62019451	受权官员 李凤云 电话号码：(86-10) **62411143**

C(续). 相关文件

类　型	引用文件，必要时，指明相关段落	相关的权利要求
X	CN1960729A(泰尔茂株式会社等)，09.5 月 2007（09.05.2007）	1-9, 11,12,14-23
Y	参见实施例 8，说明书第 9 页第 18－22 行，第 11 页 9－16 行	10, 13
X	CN101019834A(西安力邦医药科技有限责任公司)，22.8 月 2007（22.08.2007）	1-9, 11,12,14-23
Y	参见实施例 3	10, 13
X	CN101283983A(南京长澳医药科技有限公司)，15.10 月 2008（15.10.2008）	1-9, 11,15-21
Y	参见实施例 3	10
A		12-14,22,23
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(54) **Title**: COMPOSITIONS AND METHODS FOR THE TREATMENT OF CANCER

(57) **Abstract**: The present invention provides compositions and methods for the treatment of cancer are provided.

Compositions and Methods for the Treatment of Cancer

By Alexander V. Kabanov
Daria Y. Alakhova
Yi Zhao

This application claims priority under 35 U.S.C. §119(e) to U.S. Provisional Patent Application No. 61/379,882, filed on September 3, 2010. The foregoing application is incorporated by reference herein.

This invention was made with government support under Grant No. 2R01 CA89225 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates generally to cancer therapies. More specifically, the present invention provides compositions and methods for the improved delivery of therapeutic agents to a patient for the treatment of cancer.

BACKGROUND OF THE INVENTION

DOXIL® (doxorubicin HCl liposome injection) significantly reduces the side effects associated with doxorubicin. The need to administer DOXIL® only once every 4 weeks by intravenous administration also makes DOXIL® convenient for patients. However, DOXIL® has been shown to not be as effective as doxorubicin. Accordingly, means for increasing the therapeutic efficacy of DOXIL®, while preserving its safety, are desirous.

SUMMARY OF THE INVENTION

In accordance with the instant invention, methods

of inhibiting cancer in a subject are provided. In a particular embodiment, the method comprises administering at least one encapsulated chemotherapeutic agent and at least one amphiphilic block copolymer to the subject. The encapsulated chemotherapeutic agent and the amphiphilic block copolymer may be administered simultaneously and/or sequentially, particularly when the encapsulated therapeutic is administered first. In a particular embodiment, the encapsulated chemotherapeutic agent is a liposomal doxorubicin. In a particular embodiment, the amphiphilic block copolymer comprises at least one block of ethylene oxide and at least one block of propylene oxide.

In accordance with another aspect of the instant invention, compositions comprising at least one encapsulated chemotherapeutic agent, at least one amphiphilic block copolymer, and at least one pharmaceutical carrier are provided. Kits comprising a) a first composition comprising at least one encapsulated chemotherapeutic agent and at least one pharmaceutical composition; and b) a second composition comprising at least one amphiphilic block copolymer and at least one pharmaceutical composition, are also provided.

BRIEF DESCRIPTIONS OF THE DRAWING

Figures 1A and 1B show the cellular uptake of Doxil® formulations in ovarian and breast cancer cells, respectively. DOX mean fluorescence intensity was counted by flow cytometry. Cells were treated with 1) Doxil®; 2) Doxil® and 0.1% P85 co-treatment for 2 hours, 3) first treated with 0.1% P85 for 2 hours, washed three times with PBS and further incubated with Doxil® for 24 hours; 4) first treated with Doxil® for 24 hours, washed three times with PBS, followed by 2 hours treatment with

0.1% P85. Data are mean ± SEM (n = 6), * p < 0.05, n.s. stands for no significance. The p values were obtained using Student's t-test following logarithmic transformation of the data.

Figure 2 shows the nuclear uptake of Doxil® formulations in ovarian cancer cells. Cells were treated with 1) Doxil®; 2) Doxil® and 0.1% P85 co-treatment for 2 hours, 3) first treated with 0.1% P85 for 2 hours, washed three times with PBS and further incubated with Doxil® for 24 hours; 4) first treated with Doxil® for 24 hours, washed three times with PBS, followed by 2 hours treatment with 0.1% P85. After treatment the cells were washed three times with PBS, lyzed using M-PER lysis buffer and Dox fluorescence was measured using Spectamax M5 plate reader and normalized over protein content. Data are mean ± SEM (n = 6), *** p < 0.05, n.s. stands for no significance. The p values were obtained using Student's t-test following logarithmic transformation of the data.

Figure 3 provides confocal images of DOX localization in breast and ovarian cancer cell lines in the presence or absence of Pluronic® P85. Figure 3A shows Doxil® uptake in A2780 and A2780/DOX cells after 2 or 24 hours incubation, in the presence of 0.1% Pluronic® P85, Doxil® uptake in A2780 and A2780/DOX cells after 2 hours incubation. Pluronic® P85 was labeled with Atto 647. Figure 3B provides a time series imaging of post-treatment of A2780 cells with 0.1% Pluronic® P85 after 24 hours treatment with 200 µg/ml Doxil®. Figure 3C shows the Doxil®/DOX uptake in A2780 and A2780/DOX cells at different treatment regimens with 0.1% P85. The last panels in each row present digitally superimposed images of two preceding panels to visualize the colocalization. Magnification 63x.

Figure 4 provides the fluorescence spectra of free DOX and Doxil® liposome in the absence (Fig. 4A) or presence (Fig. 4B) of 0.1% Pluronic® P85.

Figure 5 provides the in vitro release profile of DOX from Doxil® mediated by P85 at 37 °C (●) pH5.5; (○) pH5.5 in 0.1% P85; (▲) pH7.4; (△) pH7.4 in 0.1% P85. Data are mean ± SEM (n = 4).

Figure 6 shows the effect of Pluronic® P85 on tumor volume in the A2780 xenografts. Doxil® alone (■) Doxil® with 1 hour post treatment of 0.02% Pluronic® P85 (○), Doxil® with 48 hours post treatment of 0.02% Pluronic® P85 (▲), Doxil® with 96 hours post treatment of 0.02% Pluronic® P85 (▽). Treatment consisted of a single i.v. injection of 12 mg/kg of Doxil® given 2 weeks after tumor implantation. The data represent mean ± SEM (n = 8), * $p < 0.05$. The p values were obtained using 2 ways ANOVA by comparing tumor volume in Doxil® group and other groups.

Figure 7 provides H&E staining of heart, liver, spleen and kidney tissues from animals in 4 treatment groups: control, Doxil®, Doxil® + P85 48 hours later, Doxil® + P85 96 hours later.

DETAILED DESCRIPTION OF THE INVENTION

DOXIL® is a PEGylated liposomal formulation of the small molecule drug doxorubicin. In a particular embodiment, DOXIL® contains 2 mg/ml of doxorubicin, 3.19 mg/ml of N-carbonylmethoxypolyethylene glycol 2000-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (DSPE-PEG 2000), 9.58 mg/ml of fully hydrogenated soy phosphatidylcholine, and 3.19 mg/ml of cholesterol. DOXIL® has been extensively used in clinics for the treatment of various types of cancers. However, DOXIL® has had only limited efficacy in these therapies.

Herein, it is shown that administering amphiphilic copolymers, such as those comprising polyoxyethylene and polyoxypropylene blocks, with liposomal doxorubicin results in substantially higher cytotoxicity of doxorubicin than in the absence of the amphiphilic block copolymer. For example, the data provided herewith shows that treatment of breast cancer cell lines MCF7, MCF7/MX and MCF7/ADR with amphiphilic block copolymer and liposomal doxorubicin is cytotoxic. However, the treatment with the liposomal doxorubicin alone was not cytotoxic at the concentrations used in the experiment when added to culture for 2 hours. Without being bound by theory, the increased cytotoxicity may be explained by significantly higher uptake of the liposomal doxorubicin into the cells in presence of or after pre-treatment with the amphiphilic block copolymer.

It is also demonstrated herein that doxorubicin release out of liposomes is stimulated when cultures were exposed to the amphiphilic block copolymer, e.g., when pretreated with the amphiphilic block copolymer or when the amphiphilic block copolymer was administered to cultures with or after liposomal doxorubicin.

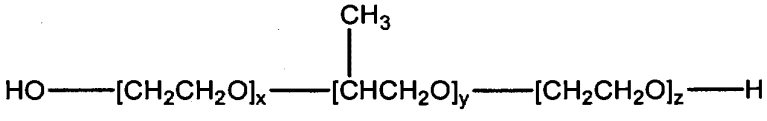
The effect of amphiphilic block copolymer administration on intracellular trafficking and uptake of liposomal doxorubicin in breast cancer cells was also evaluated. The treatment of the breast cancer cells with liposomal doxorubicin alone resulted in uptake and localization of the drug in the intracellular vesicles. When cells were treated with the liposomal doxorubicin in the presence of 0.1% amphiphilic block copolymer, the nuclear uptake of the drug was observed as early as after 15 minutes and, after 60 minutes, most of the drug was localized in the nucleus.

The instant invention encompasses methods of increasing the efficacy of an encapsulated compound (e.g., therapeutic agent or diagnostic agent) by co-administering (e.g., prior, during, and/or after) at least one amphiphilic block copolymer. The instant invention also encompasses methods of increasing the delivery of an encapsulated compound (e.g., therapeutic agent or diagnostic agent) to the nucleus of cell by co-administering (e.g., prior, during, and/or after) at least one amphiphilic block copolymer. The therapeutic agent may be encapsulated in a micelle or liposome, particularly a liposome. In a particular embodiment, the therapeutic agent acts in the nucleus (i.e., the therapeutic agent is delivered to the nucleus to exert its therapeutic effect). In a particular embodiment, the therapeutic agent is a chemotherapeutic agent. Particularly, the chemotherapeutic agent is a DNA damaging agent (see below), particularly a DNA intercalater such as an anthracycline. In a particular embodiment, the chemotherapeutic agent is doxorubicin.

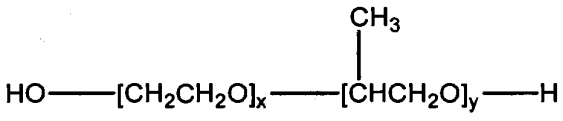
The encapsulated compound may be any bioactive agent such as a therapeutic agent or diagnostic agent. The compound may also be a test compound to be screened as potential leads in the development of therapeutic agents. Encapsulated compounds include, without limitation, polypeptides, peptides, glycoproteins, nucleic acids, synthetic and natural drugs, peptoides, polyenes, macrocyles, glycosides, terpenes, terpenoids, aliphatic and aromatic compounds, small molecules, and their derivatives and salts. In a particular embodiment, the encapsulated compound is a small molecule. While any type of compound may be delivered to a cell or subject by the compositions and methods of the instant invention, the following description of the

inventions exemplifies the compound as a therapeutic agent, particularly doxorubicin, for simplicity.

In a particular embodiment of the invention, the amphiphilic block copolymer is a copolymer comprising at least one block of poly(oxyethylene) and at least one block of poly(oxypropylene). Amphiphilic block copolymers are exemplified by the block copolymers having the formulas:



(I),



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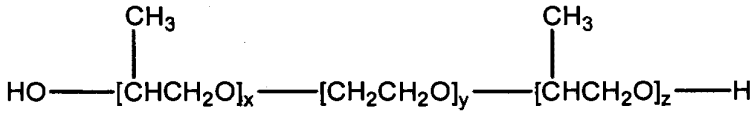

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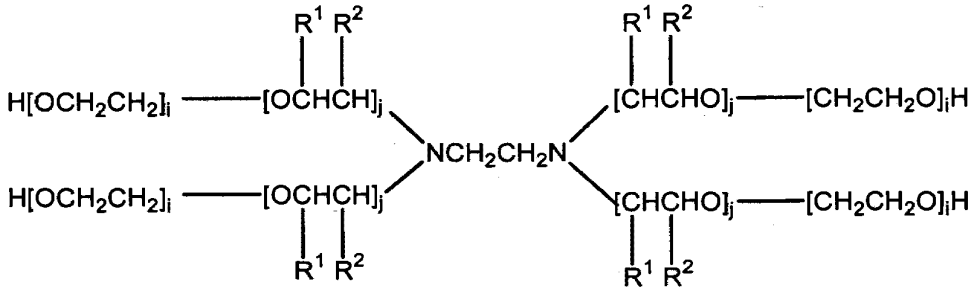
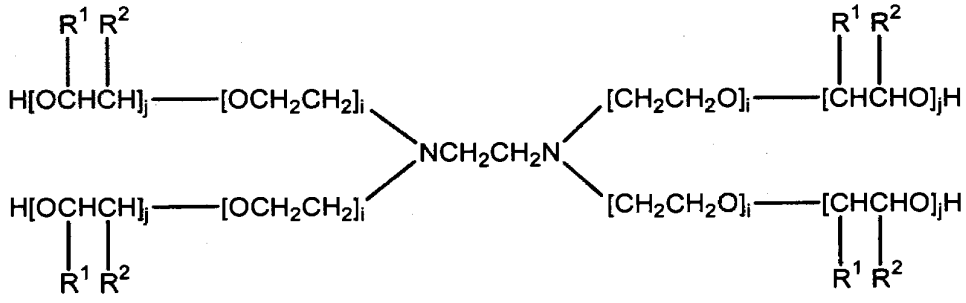


(IV),



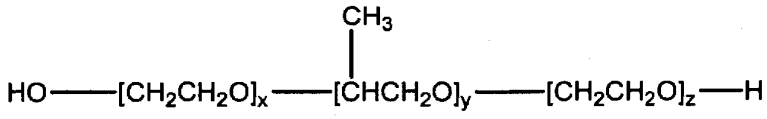
(V),

in which x, y, z, i, and j have values from about 2 to about 800, preferably from about 5 to about 200, more preferably from about 5 to about 80, and wherein for each R^1, R^2 pair, as shown in formula (IV) and (V), one is hydrogen and the other is a methyl group. The ordinarily skilled artisan will recognize that the values of x, y, and z will usually represent a statistical average and that the values of x and z are often, though not necessarily, the same. Formulas (I) through (III) are oversimplified in that, in practice, the orientation of the isopropylene radicals within the B block will be random. This random orientation is indicated in formulas (IV) and (V), which are more complete. Such poly(oxyethylene)-poly(oxypropylene) compounds have been described by Santon (Am. Perfumer Cosmet. (1958) 72(4):54-58); Schmolka (Loc. cit. (1967) 82(7):25-30), Schick, ed. (Non-ionic Suifactants, Dekker, N.Y., 1967 pp. 300-371). A number of such compounds are commercially available under such generic trade names as "lipoloxamers", "Pluronics®," "poloxamers," and "synperonics." Pluronic® copolymers within the B-A-B formula, as opposed to the A-B-A formula typical of Pluronics®, are often referred to as "reversed" Pluronics®, "Pluronic® R" or "meroxapol." Generally, block copolymers can be described in terms of having hydrophilic "A" and hydrophobic "B" block segments. Thus, for example, a copolymer of the formula A-B-A is a triblock copolymer consisting of a hydrophilic block connected to a hydrophobic block connected to another hydrophilic block. The "polyoxamine" polymer of formula (IV) is available from BASF under the tradename Tetronic®. The order of the polyoxyethylene and polyoxypropylene blocks represented in formula (IV) can be reversed, creating Tetronic R®,

also available from BASF (see, Schmolka, J. Am. Oil. Soc. (1979) 59:110).

Polyoxypropylene-polyoxyethylene block copolymers can also be designed with hydrophilic blocks comprising a random mix of ethylene oxide and propylene oxide repeating units. To maintain the hydrophilic character of the block, ethylene oxide can predominate. Similarly, the hydrophobic block can be a mixture of ethylene oxide and propylene oxide repeating units. Such block copolymers are available from BASF under the tradename Pluradot™. Poly(oxyethylene)-poly(oxypropylene) block units making up the first segment need not consist solely of ethylene oxide. Nor is it necessary that all of the B-type segment consist solely of propylene oxide units. Instead, in the simplest cases, for example, at least one of the monomers in segment A may be substituted with a side chain group.

A number of poloxamer copolymers are designed to meet the following formula:



(I).

Examples of poloxamers include, without limitation, Pluronic® L31, L35, F38, L42, L43, L44, L61, L62, L63, L64, P65, F68, L72, P75, F77, L81, P84, P85, F87, F88, L92, F98, L101, P103, P104, P105, F108, L121, L122, L123, F127, 10R5, 10R8, 12R3, 17R1, 17R2, 17R4, 17R8, 22R4, 25R1, 25R2, 25R4, 25R5, 25R8, 31R1, 31R2, and 31R4. Pluronic® block copolymers are designated by a letter prefix followed by a two or a three digit number. The letter prefixes (L, P, or F) refer to the physical

form of each polymer, (liquid, paste, or flakeable solid). The numeric code defines the structural parameters of the block copolymer. The last digit of this code approximates the weight content of EO block in tens of weight percent (for example, 80% weight if the digit is 8, or 10% weight if the digit is 1). The remaining first one or two digits encode the molecular mass of the central PO block. To decipher the code, one should multiply the corresponding number by 300 to obtain the approximate molecular mass in daltons (Da). Therefore Pluronic nomenclature provides a convenient approach to estimate the characteristics of the block copolymer in the absence of reference literature. For example, the code 'F127' defines the block copolymer, which is a solid, has a PO block of 3600 Da (12X300) and 70% weight of EO. The precise molecular characteristics of each Pluronic® block copolymer can be obtained from the manufacturer.

In a particular embodiment, the amphiphilic block copolymer is a PEO-PPO-PEO triblock copolymer. In a particular embodiment, the PEO-PPO-PEO triblock copolymer comprises about 15 to about 35, particularly about 20 to about 30 PEO monomers at each end and a center block of about 30 to about 50, particularly about 35 to about 45 PPO monomers. In one embodiment, the PEO/PPO/PEO monomer unit ratio is 26/40/26. In a particular embodiment, the amphiphilic block copolymer is poloxamer 235.

The amphiphilic block copolymers may have a PPO content of at least 30% wt, at least 40% wt, or at least 50% wt. The amphiphilic block copolymers may have a PPO block molecular mass of at least about 1200 or 1700 to about 3000, 3600, or 4200. In a particular embodiment, the amphiphilic block copolymer is a PEO-PPO-PEO

triblock copolymer with a PPO content of at least 30% wt and a PPO block molecular mass from about 1200 to about 4200; a PPO content of at least 40% wt and a PPO block molecular mass from about 1700 to about 3600; or, more particularly, a PPO content of at least 50% wt and a PPO block molecular mass from about 1700 to about 3000.

As stated hereinabove, more than one block copolymer may be used. In other words, a mixture or blend of block copolymers may be used. For example, the mixture or blend may comprise at least one PEO-PPO-PEO triblock copolymer. For example, the mixture may comprise at least one first amphiphilic block copolymer and at least one second amphiphilic block copolymer. In a particular embodiment, (1) the first amphiphilic block copolymer is a PEO-PPO-PEO triblock copolymer with a PPO content of at least 30% and the second amphiphilic block copolymer is PEO-PPO-PEO triblock copolymer with a PPO content of 70% or less; (2) the first amphiphilic block copolymer is a PEO-PPO-PEO triblock copolymer with a PPO content of at least 40% and the second amphiphilic block copolymer is a PEO-PPO-PEO triblock copolymer with a PPO content of 60% or less; (3) the first amphiphilic block copolymer is a PEO-PPO-PEO triblock copolymer with a PPO content of at least 50% and the second amphiphilic block copolymer is a PEO-PPO-PEO triblock copolymer with a PPO content of 50% or less; (4) the first amphiphilic block copolymer is a PEO-PPO-PEO triblock copolymer with a PPO content of at least 60% and the second amphiphilic block copolymer is a PEO-PPO-PEO triblock copolymer with a PPO content of 40% or less; or (5) the first amphiphilic block copolymer is a one PEO-PPO-PEO triblock copolymer with a PPO content of at least 70% and the second amphiphilic block copolymer is a PEO-PPO-PEO triblock copolymer with a PPO content of 30% or less. In the

case of mixtures, the PEO-PPO-PEO block copolymers, independently of each other, may have a PPO block molecular mass from about 900 to about 4200, from about 1700 to about 3600, or, more particularly, from about 1700 to about 3000.

Administration

The instant invention encompasses compositions comprising at least one amphiphilic block copolymer, at least one encapsulated therapeutic agent (e.g., liposomal doxorubicin), and at least one pharmaceutically acceptable carrier. The instant invention also encompasses comprising a first composition comprising at least one amphiphilic block copolymer and at least one pharmaceutically acceptable carrier and a second composition comprising at least one encapsulated therapeutic agent and at least one therapeutic agent. The compositions of the instant invention may further comprise other therapeutic agents (e.g., other chemotherapeutic agents).

The present invention also encompasses methods for preventing, inhibiting, and/or treating a disease or disorder (e.g., a cancer/neoplasia) in a subject. The methods comprise administering at least one amphiphilic block copolymer and at least one encapsulated therapeutic agent (related to the disease or disorder) to the subject. The pharmaceutical composition(s) of the instant invention can be administered to an animal, in particular a mammal, more particularly a human, in order to treat/inhibit cancer. The amphiphilic block copolymer may be administered in the same composition as the encapsulated therapeutic agent or in different compositions. The amphiphilic block copolymer may be administered concurrently and/or sequentially with the

encapsulated therapeutic agent. For example, the amphiphilic block copolymer may be administered to the subject prior to the administration of the encapsulated therapeutic agent, may be co-administered with the encapsulated therapeutic agent, and/or may be administered after the administration of the encapsulated therapeutic agent.

In a particular embodiment, the amphiphilic block copolymer is administered after the encapsulated therapeutic agent (liposomal doxorubicin). In a particular embodiment, the amphiphilic block copolymer is administered within 96 hours, within 72 hours, within 48 hours, within 24 hours, within 12 hours, within 6 hours, within 4 hours, within 2 hours, within 1 hour, within 0.5 hour, or less after the administration of the encapsulated therapeutic. In a particular embodiment, the amphiphilic block copolymer is administered about 1 hour to about 48 hours, particularly 24 hours after administration of the encapsulated therapeutic.

The amphiphilic block copolymer may be present in the composition at any concentration. In a particular embodiment, the amphiphilic block copolymer is present at a concentration from about 0.001% to about 5%. In a particular embodiment, the concentration of the amphiphilic block copolymer is from about 0.05% to about 2%.

In a particular embodiment, the therapeutic agent is a chemotherapeutic agent. The term "chemotherapeutic agent" means anti-cancer and other anti-hyperproliferative agents. Chemotherapeutic agents are compounds that exhibit anticancer activity and/or are detrimental to a cell. Chemotherapeutic agents include, without limitation: (1) DNA damaging agents (e.g., agents that inhibit DNA synthesis): anthracyclines

(e.g., doxorubicin, daunorubicin, epirubicin), alkylating agents (e.g., nitrogen mustards, bendamustine, altretamine, methanesulphonate esters, busulfan, carboplatin, nitroso ureas, carmustine, cisplatin, chlorambucil, cyclophosphamide, dacarbazine, hexamethylmelamine, isofamide, ifosphamide, lomustine, mechlorethamine, alkyl sulfonates, epirubicin, idarubicin, triazines, ethylenimines, melphalan, mitotane, mytomycin, pipobroman, procarbazine, streptozocin, aziridines, thiotepa, uracil mustard, and triethylenemelamine), platinum derivatives (e.g., cisplatin, carboplatin, tetraplatin, ormaplatin, thioplatin, satraplatin, nedaplatin, oxaliplatin, heptaplatin, iproplatin, transplatin, lobaplatin, and cis diamminedichloro platinum), telomerase and topoisomerase inhibitors (e.g., topotecan, irinotecan, etoposide, teniposide, amsacrine, menogaril, amonafide, dactinomycin, daunorubicin, N,N-dibenzyl daunomycin, ellipticine,, daunomycin, pyrazoloacridine, idarubicin, mitoxantrone, m-ANSA, doxorubicin, deoxyrubicin, oxanthrazole, rubidazone, epirubicin, and bleomycin), DNA minor groove binding agents (e.g., plicamydin); (2) tubulin interactive agents (e.g., vincristine, vinblastine, paclitaxel, taxoids, paclitaxel, docetaxel, and BAY 59-8862); (3) anti-metabolites such as capecitabine, chlorodeoxyadenosine, cytarabine, ara-CMP, cytosine arabinoside, asparginase, azacitidine, dacabazine, floxuridine, fludarabine, 5-fluorouracil, 5-DFUR, gemcitibine, hydroxyurea, 6-mercaptopurine, methotrexate, pentostatin, pemetrexed, trimetrexate, and 6-thioguanine; (4) anti-angiogenics (e.g., Avastin, thalidomide, sunitinib, lenalidomide) and vascular disrupting agents (e.g., flavonoids/flavones, DMXAA, combretastatin derivatives such as CA4DP, ZD6126,

AVE8062A); (5) antibodies or antibody fragments (e.g., trastuzumab, bevacizumab, rituximab, ibritumomab, gemtuzumab, alemtuzumab, cetuximab, ranibizumab); and (6) hormonal agents/endocrine therapy: aromatase inhibitors (e.g., 4-hydroandrostendione, exemestane, aminoglutehimide, anastrozole, letozole), anti-estrogens (e.g., Tamoxifen, Toremifine, Raoxifene, Faslodex), anti-androgen agents (e.g., flutamide), anti-adrenal agents (e.g., mitotane and aminoglutethimide), and steroids (e.g., adrenal corticosteroids, prednisone, dexamethasone, methylprednisolone, and prednisolone); and (7) anti-mitotic compounds such as navelbine, epothilones, taxanes (e.g., paclitaxel, taxotere, docetaxel), vinca alkaloids, estramustine, vinblastine, vincristine, vindesine, and vinorelbine).

Cancers that may be treated using the present protocol include, but are not limited to: cancers of the prostate, colorectum, pancreas, cervix, stomach, endometrium, brain, liver, bladder, ovary, testis, head, neck, skin (including melanoma and basal carcinoma), mesothelial lining, white blood cell (including lymphoma and leukemia) esophagus, breast, muscle, connective tissue, lung (including small-cell lung carcinoma and non-small-cell carcinoma), adrenal gland, thyroid, kidney, or bone; glioblastoma, mesothelioma, renal cell carcinoma, gastric carcinoma, sarcoma, choriocarcinoma, cutaneous basocellular carcinoma, skin squamous cell carcinomas, and testicular seminoma. In a particular embodiment, the cancer is selected from the group consisting of ovarian cancer, lung cancer including small-cell and non-small cell lung cancer, gastric cancer, breast cancer, Kaposi's sarcoma, uterine cancer, hematological cancer including multiple myeloma,

lymphoma, Hodgkin's lymphoma and Non-Hodgkin's lymphoma and ovarian cancer.

The composition(s) described herein will generally be administered to a patient as a pharmaceutical preparation. The term "patient" as used herein refers to human or animal subjects. These compositions may be employed therapeutically, under the guidance of a physician.

The compositions of the instant invention may be conveniently formulated for administration with any pharmaceutically acceptable carrier(s). For example, the agents may be formulated with an acceptable medium such as water, buffered saline, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like), dimethyl sulfoxide (DMSO), oils, detergents, suspending agents or suitable mixtures thereof. The concentration of the agents in the chosen medium may be varied and the medium may be chosen based on the desired route of administration of the pharmaceutical preparation. Except insofar as any conventional media or agent is incompatible with the agents to be administered, its use in the pharmaceutical preparation is contemplated. Notably, formulations and dosages of liposomal doxorubicin (DOXIL®) are well known in the art (see, e.g., DOXIL® Product Information (2010) Centocor Ortho Biotec Products).

The dose and dosage regimen of compositions according to the invention that are suitable for administration to a particular patient may be determined by a physician considering the patient's age, sex, weight, general medical condition, and the specific condition for which the composition is being administered and the severity thereof. The physician may also take into account the route of administration,

the pharmaceutical carrier, and the composition's biological activity.

Selection of a suitable pharmaceutical preparation will also depend upon the mode of administration chosen. For example, the compositions of the invention may be administered by direct injection to a desired site (e.g., a tumor). In this instance, a pharmaceutical preparation comprises the agents dispersed in a medium that is compatible with the site of injection. Compositions of the instant invention may be administered by any method. For example, the compositions of the instant invention can be administered, without limitation parenterally, subcutaneously, orally, topically, pulmonarily, rectally, vaginally, intravenously, intraperitoneally, intrathecally, intracerbrally, epidurally, intramuscularly, intradermally, or intracarotidly. In a particular embodiment, the compositions are administered by injection, such as intravenously or intraperitoneally. Pharmaceutical preparations for injection are known in the art. If injection is selected as a method for administering the composition, steps must be taken to ensure that sufficient amounts of the molecules reach their target cells to exert a biological effect.

Pharmaceutical compositions containing an agent of the present invention as the active ingredient in intimate admixture with a pharmaceutically acceptable carrier can be prepared according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., intravenous or direct injection.

In a particular embodiment, the encapsulated therapeutic agent (e.g., liposomal doxorubicin) is administered via injection, particularly intravenous injection. In a particular embodiment, the amphiphilic block copolymer is administered via injection, e.g., intravenous, subcutaneous, or intramuscular.

A pharmaceutical preparation of the invention may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form, as used herein, refers to a physically discrete unit of the pharmaceutical preparation appropriate for the patient undergoing treatment. Each dosage should contain a quantity of active ingredient calculated to produce the desired effect in association with the selected pharmaceutical carrier. Procedures for determining the appropriate dosage unit are well known to those skilled in the art.

Dosage units may be proportionately increased or decreased based on the weight of the patient. Appropriate concentrations for alleviation of a particular pathological condition may be determined by dosage concentration curve calculations, as known in the art.

In accordance with the present invention, the appropriate dosage unit for the administration of compositions of the instant invention may be determined by evaluating the toxicity of the molecules or cells in animal models. Various concentrations of agents in pharmaceutical preparations may be administered to mice, and the minimal and maximal dosages may be determined based on the beneficial results and side effects observed as a result of the treatment. Appropriate dosage unit may also be determined by assessing the efficacy of the agent treatment in combination with

other standard drugs. The dosage units of the compositions may be determined individually or in combination with each treatment according to the effect detected.

The pharmaceutical preparation comprising the agents of the instant invention may be administered at appropriate intervals, for example, at least twice a day or more until the pathological symptoms are reduced or alleviated, after which the dosage may be reduced to a maintenance level. The appropriate interval in a particular case would normally depend on the condition of the patient.

Other methods of treating cancer may be combined with the methods of the instant invention. For example, other chemotherapeutic agents may be administered (e.g., simultaneously and/or consecutively). Cancer therapies such as radiation and/or surgery (e.g., tumor excision) may also be co-administered with the compositions of the instant invention.

Definition

"Pharmaceutically acceptable" indicates approval by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

A "carrier" refers to, for example, a diluent, adjuvant, preservative (e.g., Thimersol, benzyl alcohol), anti-oxidant (e.g., ascorbic acid, sodium metabisulfite), solubilizer (e.g., Tween 80, Polysorbate 80), emulsifier, buffer (e.g., Tris HCl, acetate, phosphate), antimicrobial, bulking substance (e.g., lactose, mannitol), excipient, auxiliary agent or vehicle with which an active agent of the present

invention is administered. Pharmaceutically acceptable carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin. Water or aqueous saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin (Mack Publishing Co., Easton, PA); Gennaro, A. R., Remington: The Science and Practice of Pharmacy, (Lippincott, Williams and Wilkins); Liberman, et al., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y.; and Kibbe, et al., Eds., Handbook of Pharmaceutical Excipients, American Pharmaceutical Association, Washington.

The term "treat" as used herein refers to any type of treatment that imparts a benefit to a patient afflicted with a disease, including improvement in the condition of the patient (e.g., in one or more symptoms), delay in the progression of the condition, etc. In a particular embodiment, the treatment of a retroviral infection results in at least an inhibition/reduction in the number of infected cells.

A "therapeutically effective amount" of a compound or a pharmaceutical composition refers to an amount effective to prevent, inhibit, treat, or lessen the symptoms of a particular disorder or disease. The treatment of cancer herein may refer to curing, relieving, and/or preventing cancer, the symptom(s) of it, or the predisposition towards it.

As used herein, the term "therapeutic agent" refers to a chemical compound or biological molecule including, without limitation, nucleic acids, peptides, proteins, and antibodies that can be used to treat a condition,

disease, or disorder or reduce the symptoms of the condition, disease, or disorder.

As used herein, the term "small molecule" refers to a substance or compound that has a relatively low molecular weight (e.g., less than 4,000, less than 2,000, particularly less than 1 kDa or 800 Da). Typically, small molecules are organic, but are not proteins, polypeptides, or nucleic acids, though they may be amino acids or dipeptides.

As used herein, the term "amphiphilic" means the ability to dissolve in both water and lipids/apolar environments. Typically, an amphiphilic compound comprises a hydrophilic portion and a hydrophobic portion. "Hydrophobic" designates a preference for apolar environments (e.g., a hydrophobic substance or moiety is more readily dissolved in or wetted by non-polar solvents, such as hydrocarbons, than by water). As used herein, the term "hydrophilic" means the ability to dissolve in water.

As used herein, the term "polymer" denotes molecules formed from the chemical union of two or more repeating units or monomers. The term "block copolymer" most simply refers to conjugates of at least two different polymer segments, wherein each polymer segment comprises two or more adjacent units of the same kind.

The following example provides illustrative methods of practicing the instant invention, and is not intended to limit the scope of the invention in any way.

EXAMPLE

Doxil®, a PEGylated liposomal formulation of small molecular anticancer drug, doxorubicin (DOX), has been

extensively used in clinics for the treatment of various types of cancers (e.g., ovarian cancer, AIDS-Related Kaposi's sarcoma and multiple myeloma) (Amantea et al. (1997) Clin. Pharmacol. Ther., 61:301-311; Sharpe et al. (2002) Drugs 62:2089-2126; Gabizon et al. (2001) Cancer Invest., 19:424-436). However, the response rate of Doxil® single agent is limited and the combinations of Doxil® with other chemotherapeutic drugs (Taxol®, Hycamtin®) have been reported to be well tolerated by patients and have higher efficacy (Campos et al. (2003) Gynecol. Oncol., 90:610-618; Dunton, C.J. (1997) Semin. Oncol., 24:S5-2-S5-11). Thus, the studies of new Doxil® combination with other agents are of increasing interest for development of new cancer treatments. The amphiphilic block copolymers, Pluronics®, consisting of polyoxyethylene-polyoxypropylene blocks, were used in the instant study with several regimens of combinational therapies: pretreatment with block copolymers with subsequent administration of Doxil®, co-administration of Doxil® and Pluronic®, and Doxil® treatment with consequent post-treatment with Pluronic®. The cytotoxicity studies in ovarian cancer sensitive A2780 and resistant A2780/DOX as well as breast cancer sensitive and resistant cell lines, MCF7, MCF7/ADR, in vitro data indicates that co-administration with Doxil® pre-treatment or post-treatment cells with Pluronic® at nontoxic concentration, induced significant more nuclear uptake of DOX than Doxil® alone. DOX in vitro release profiles from Doxil® liposomes and fluorescence quenching analysis have revealed that Pluronic® induces the increase of mobility and permeability of liposomal lipid bilayer, which may attribute to the release of the drug from liposomes. Pluronic® 1 hour and 48 hours post

treatments with Doxil® have shown significantly better antitumor activities than Doxil® alone group.

Combinations of well-characterized drugs that have been successfully used in clinics for the treatment of cancer patients with novel materials/activators, and use of novel treatment regimens may serve a solution and offer substantial advantages as compared to therapeutic molecules used alone. Doxil®, a PEGylated liposomal formulation of the anticancer drug doxorubicin, has been extensively used in phase I and phase II combination studies for the treatment of various types of cancers. The response rates of these combination, particularly in platinum-resistant ovarian cancer, were reported highly than single-agent PEGylated liposome doxorubicin (Rose et al. (2008) Am. J. Clin. Oncol., 31:476-480; Markman et al. (2004) Semin. Oncol., 31:91-105; Gabizon et al. (1994) Acta Oncol., 33:779-786; Eltabbakh et al. (2001) Expert Opin. Pharmacother., 2:109-124). However, the combination therapy is not deprived of side effects and the administration of more than one cytotoxic agent can result in even more severe systemic toxicity of the formulation in comparison to single agents. An ideal agent for combination therapy would have low systemic toxicity and have synergistic effect with the other drug in the formulation. Combinations of liposomal doxorubicin with ATP sensitizers for ovarian cancer have been reported (Di Nicolantonio et al. (2002) Anticancer Drugs, 13:625-630; Cree, I.A. (2003) Cancer Res., 161:119-125; Knight et al. (2009) BMC Cancer, 9:38). Pluronic® block copolymers are very potent chemosensitizer of mulidrug resistant (MDR) cancers (Batrakova et al. (2010) J. Control Release, 143:290-301; Batrakova et al. (2003) Pharm. Res., 20:1581-1590). Pluronic® block copolymers inhibit P-pg ATPase activity,

a major protein associated with multidrug resistance in numerous cancers, responsible for efflux of cytotoxic drugs from the cells (Kabanov et al. (2003) J. Control Release, 91:75-83). Additionally, Pluronic® induces ATP depletion, inhibition of oxygen consumption, and inhibition of mitochondria respiratory chain complexes I and IV selectively in MDR cells. All together these effects significantly increase the cytotoxicity of doxorubicin in drug resistant cancers (Alakhova et al. (2010) J. Control Release, 142:89-100). Notably, SP1049C, the formulation of Pluronics® L61, F127 with doxorubicin has successfully completed Phase II clinical trial in advanced esophageal adenocarcinoma (Batrakova et al. J. Control Release, 130:98-106). Here, a novel therapeutic approach is provided for the treatment of cancer using a combination of liposomal doxorubicin, Doxil®, with Pluronic® block copolymer, and a treatment regimen that drastically enhances the anticancer efficiency of Doxil®.

Materials and Methods

Chemicals and materials

Doxil® (DOX HCl liposome injection) was purchased from ALZA Corp (Mountain View, CA). ATP assay kit (#FLAA-1KT), Thiazolyl Blue Tetrazolium Bromide (MTT, #M5655-1G), Dulbecco's phosphate buffered saline solution (PBS) were purchased from Sigma-Aldrich (St. Louis, MO). Pluronic® P85 (lot # WPYE537B) was kindly provided by BASF Corporation (North Mount Olive, NJ). A PEO/PPO/PEO monomer unit ratio is 26/40/26 in P85. Assay buffer is 122 mM sodium chloride, 25 mM sodium bicarbonate, 10 mM glucose, 10 mM HEPES, 3 mM potassium chloride, 1.2 mM magnesium sulfate, 1.4 mM calcium

chloride and 0.4 mM potassium phosphate dibasic and adjusted to pH 7.4.

Cells and culture conditions

A2780, MCF7 were purchased from ATCC. A2780 and A2780/DOX were cultured in RPMI 1640 media, MCF7 and MCF7/ADR were cultured in DMEM media, with 10% Fetal Bovine Serum (Invitrogen, Carlsbad, CA), 100 U/ml penicillin, 100 mg/ml streptomycin. MCF7/ADR, A2780/DOX were cultured in the presence of 1 μg/ml DOX. Cells were grown at 37°C in a humidified atmosphere of 5% CO_2 (v/v) in air. All experiments were performed on cells in the exponential growth phase.

Western blot assay

To identify Pgp phenotype of cells, immunoblots were performed. The monoclonal antibodies to Pgp, C219 (Dako Corp. Carpinteria, CA) were used at 1:100 dilutions. The monoclonal antibody to β-actin, (Sigma Inc.) was used with a 1:15000 dilution. The secondary horseradish peroxide anti-mouse Ig antibodies (1:20000 dilution) was purchased from Sigma Inc.

Cytotoxicity Assay

Cells were seeded in 96-well plates at an initial density of 8×10^3 cells/well 24 hours prior to treatment. On the following day, cells were treated with DOX or Doxil® with or without 0.1% (w/v) P85 for 2 or 24 hours at 37°C in a humidified, 5% CO_2 atmosphere. Following treatment, the medium was removed and the cells were rinsed three times by PBS and cultured for three days in fresh medium. The cytotoxicity was evaluated using a standard MTT assay and the absorbance was determined at 562 nm using Spectra MX M5®. Each

concentration point was determined from samples from eight separate wells. IC_{50} values were calculated based on the percentage ratio between treated cells and untreated control by using GraphPad Prism 5 Software (GraphPad Software, San Diego Califonia).

Cellular uptake assay

Cells were seeded on 24 well-plate with the initial density of 8×10^3 cells/well, on the day of treatment, the cells were 70-80% confluent. After 2 or 24 hours incubation with or without 0.1% (w/v) Pluronic® P85, cells were rinsed three times with PBS, trypsinized, supplemented with 1 ml of complete media, and collected by centrifugation at 1500 rpm for 3 minutes. The cell pellet was re-suspended in 1ml of PBS containing 1% BSA and analyzed for DOX fluorescence in UNMC cell analysis core facility.

Confocal images

Cells were seeded 48 hrs prior treatments in 8-well chamber slides with an initial cell density of 1.0×10^4. Confluent cells were exposed to Doxil® (200 or 400 µg/ml) for 24 or 48 hours to identify the intracellular localization of DOX. Cell nuclei were additionally stained by Hoechst 33258 (Sigama, St. Louis, MO). Atto 647 labeled Pluronic® P85 was synthesized as described (Yi et al. (2010) Free Radic. Biol. Med., 49:548-558). 0.007% Atto 647 labeled P85 mixed with 0.1% unlabeled P85 were used to investigate the colocalization of Pluronic® and cytoplasm. Cells were then visualized utilizing live cell confocal imaging (Carl Zeiss LSM 510 Meta, Peabody, MA).

Release studies of Doxil®

Effect of P85 on release of DOX from Doxil® was studied by dialysis method using a membrane (MW cut-off 3,500 Da). 1 ml of Doxil® solution with DOX concentration of 0.2 mg/ml in phosphate buffered saline were placed into a dialysis bags and dialyzed against 25 ml of PBS under continuous shaking at 37°C in dark. 1 ml samples of the dialysate solution were withdrawn at a definite time interval (1, 2, 4, 8 and 24 hours) and replaced with an equal volume of fresh media. The concentration of DOX in the dialysate samples was determined by measuring absorbance at 485 nm using Lambda 25 UV/VIS spectrophotometer. The amount of DOX released from Doxil® was expressed as a percentage of the total DOX and plotted as a function of time.

Fluorescence studies

Fluorescence spectra of free DOX and Doxil® liposome in the presence of 0.1% Pluronic® P85 were recorded using a spectrofluorometer system (Flourlog®, HORIBA Jobin Yvon Inc., NJ) at wavelength of 480 nm with the bandwidth of 5 nm of excitation and emission. Solutions of free DOX and Doxil® used for these studies were at 50 μM equivalent concentrations of DOX. Fluorescence measurement for Tritc-labeled P85 was performed at λ_{ex} = 550 nm with the bandwidth of 2 nm for excitation and emission.

Size and ζ-potential measurements

Zetasizer (Marvern Instruments Limited. U.K) was used to determine effective hydrodynamic diameters (Deff) and zeta-potential of the particles at 25°C. Software provided by the manufacturer was used to analyze the size of the particles, polydispersity indices and zeta-potential of liposomes. The mean

values were calculated from the measurements performed at least in triplicate.

Animals

All experiments were carried out with the approval of the University of Nebraska Medical Center Institutional Animal Care and Use Committee and in accordance with the NIH Guide for Laboratory Animal Use. Athymic nu/nu mice (6- to 8-weeks-old females, National Cancer Institute, Frederick, MD) were used to generate the tumor model throughout this study. The animals were kept in groups of five and fed *ad libitum*.

Animal tumor model and antitumor activity

Human ovarian carcinoma xenografts were used as previous described (Pakunlu et al. (2006) J. Control Release, 114:153-162; Wang et al. (2008) Clin. Cancer Res., 14:3607-3616). A2780 human ovarian cancer cells (4.0×10^6) were subcutaneously (s.c.) injected into the right flanks of female athymic nu/nu mice. When the tumors reached a size of about 0.5 cm^3 (10-15 days after transplantation), mice were given intravenous (i.v.) injections via the tail vein with Doxil® (12 mg DOX/kg for the single injection). In the post treatment groups, 1 hour or 48 hours later, the mouse were treated with 0.02% (w/v) P85 as the same volume of Doxil® as they were administrated before. Animal weight and tumor volume was measured every other day. The tumor length (L) and width (W) were calculated by equation: $WR=1/2 \times L \times W^2$.

Statistical analysis

The differences between treatment groups were analyzed by using student's t-test for pairs of groups

and one-way analysis of variance (ANOVA) for multiple groups. The p-value less than 0.05 were considered statistically significant. All statistical analyses were carried out using GraphPad Prism Software (Version 5.0, GraphPad Software, San Diego California, USA).

Results

Effect of Pluronic on in vitro cytotoxicity of Doxil® cancer cells

For the majority of the studies, the sensitive and multidrug resistant (MDR) ovarian cancer cells, A2780 and A2780/DOX were used. Selected studies were also done using sensitive and MDR breast adenocarcinoma cells, MCF7 and MCF7/ADR. First, cells were exposed to increasing concentrations of Doxil® for 2 hours in the presence or absence of P85 (0.1% w/v). As shown in Table 1, at 2 hours exposure Doxil® alone at concentrations up to 200 mg/ml (counting per Dox contained in the liposome formulation) did not induce cytotoxicity in either ovarian cancer cell line. However, co-treatment with P85 increased Doxil® cytotoxicity in both sensitive and resistant cancer cells. 2 hours exposures to 0.1% P85 alone did not induce cytotoxicity in the same cells. Similar results were obtained for breast cancer cells (Table 2).

Treatment	Doxil® IC_{50}, µg/ml [a]	
	A2780	A2780/DOX
Doxil® (2 hrs)	N.D.[*]	N.D.[e]
Doxil® + 0.1% P85 (2 hrs, co-exposure) [b]	7.55 ± 0.36	8.32 ± 0.56
Doxil® (24 hrs)	48.02 ± 4.85	N.D.[e]
Doxil® (24 hrs) + 0.1% P85 (2 hrs, pre-exposure) [c]	17.48 ± 1.14 [(**)]	N.D.[e]
Doxil® (24 hrs) + 0.1% P85 (2 hrs, post-exposure) [d]	14.76 ± 3.24 [(**)]	N.D.[e]

Table 1: IC_{50} values (µg/mL) of Doxil® in ovarian cancer cells upon different treatments. [a] Experiments were performed in quadruplicate and data is expressed as means ± SEM of at least 4 independent experiments. N.D. non-detectable up to 200 µg/ml. Statistical comparisons were made by t-test between Doxil® (24 hours) and either of the pre- or post-exposure groups: ** $p < 0.05$. [b] Cells were co-incubated for 2 hours with Doxil® and 0.1% P85, washed three times with PBS, and grown in fresh medium for 72 hours before measuring cytotoxicity. [c] Cells were first treated with 0.1% P85 for 2 hours, washed three times with PBS, further incubated with Doxil® for 24 hours, washed three times with PBS, and grown in fresh medium for 72 hrs before measuting cytotoxicity. [d] Cells were first treated with Doxil® for 24 hours, washed three times with PBS, followed by 2 hours treatment with 0.1% P85.

Treatment	Doxil® IC_{50}, µg/ml [a]	
	MCF7	MCF7/ADR
Doxil® (2 hrs)	N.D.*	N.D.[e]
Doxil® + 0.1% P85 (2 hrs, co-exposure) [b]	43.24 ± 0.38	52.49 ± 0.73
Doxil® (24 hrs)	6.24 ± 0.26	N.D.
Doxil® (24 hrs) + 0.1% P85 (2 hrs, pre-exposure) [c]		N.D.
Doxil® (24 hrs) + 0.1% P85 (2 hrs, post-exposure) [d]		N.D.

Table 2: IC_{50} values (µg/mL) of Doxil® in breast cancer cells upon different treatments. See Table 1 for description.

The effects P85 on Doxil® cytotoxicity was examined upon 24 hours exposure. To avoid P85 toxicity the copolymer was added to cells for 2 hours only either immediately before or after exposure to Doxil®. This also allowed for the exclusion of the direct interaction between P85 and Doxil® in the exposure media. Doxil® alone, at 24 hours exposure displayed toxicity in sensitive but not resistant cell lines (Tables 1 and 2). The pretreatment and post treatment with P85 also increased cytotoxicity of Doxil® in A2780 cell line, compared to Doxil® alone group.

Effect of Pluronic® on drug uptake in cancer cells

Intracellular accumulation of Doxil® was examined by FACS analysis. This method did not allow for distinguishing between Dox incorporated into or released from the Doxil® particles, but provided a good measure of total Dox fluorescence in cells. Cells were exposed to Doxil® for 2 hours or 24 hours. P85 was added exactly as in the cytotoxicity experiments described above: either concurrently with Doxil® in the case of 2 hours exposures, or for 2 hours before or after Doxil® in the case of 24 hours exposures. In each case, except for pre-exposure in MDR cells, 0.1% P85 resulted in significant increases in drug intracellular accumulation (Figs. 1A and 1B). In a similar experiment, cells were lyzed and Dox fluorescence was normalized to the amount of cell protein. The overall results were similar to the FACS study (Fig. 2), which demonstrates that results are changes in the net uptake of the drug rather than its re-distribution inside cells between the free and liposome bound forms, which have different fluorescence.

The P85 induced increases in the drug uptake were most pronounced in the case of co-treatment, when both the copolymer and Doxil® were present in the cell culture medium simultaneously. This indicated that P85 co-exposure with Doxil® promoted the release of the drug from the liposome. This trend was seen in both sensitive and MDR cells. It is interesting to note, that in the MDR cells the levels of the drug uptake were considerably less than those in sensitive cells (Fig. 1A and 1B). This may be due to either lower rate of internalization of Doxil® particles or rapid efflux of free Dox released from the liposomes internalized inside the cells. Since, P85 is a well-documented inhibitor of the P-glycoprotein (Pgp) efflux pump, it is likely that

one of its activities included inhibition of Dox efflux in the MDR cells. This was most likely to happen in the conditions of the co-treatment of Doxil® with P85, which were most favorable for the copolymer's induced drug release and efflux inhibition. As a result under these conditions, the differences in uptake between sensitive and resistant cells were greatly reduced. The conditions of pre-treatment with P85 after 24 hours exposure to Doxil® appeared to be less likely to affect drug release and efflux, which may explain lower P85 effect of drug uptake in MDR cells.

Cellular trafficking of Doxil® in cancer cells in the absence and presence of Pluronic®

Intracellular localization of drugs plays crucial role in their activity and toxicity. DOX works via intercalation into DNA and inhibition of topoisomerase II, which unwinds DNA for transcription and thus stopping the replication process (Gewirtz, D.A. (1999) Biochem. Pharmacol., 57:727-741). Therefore it is important that DOX reaches the nucleus. Uptake of liposomal DOX, Doxil®, is rather slow process and after 24 hours of exposure to 200 μg/ml Doxil® the uptake is low and DOX fluorescence is seen in intracellular vesicles with no nuclear localization (Fig. 3A). However, 0.1% Pluronic® P85 together with Doxil® drastically increased the DOX cellular uptake and nuclear translocation in both drug sensitive and resistant cells (Fig. 3A). In the presence of 0.1% Pluronic® the drug uptake by the cells was observed as early as after 5 minutes and is quickly translocated to the nucleus (Fig. 3B). Intracellular localization of P85 was observed using Atto647 labeled Pluronic. The

cells were treated with 0.007% of labeled polymer supplemented with 0.1% of unlabeled Pluronic® (Fig. 3A).

The effect of post-treatment with 0.1% P85 on Doxil®/DOX trafficking was further studied in A2780 cells (Fig. 3C). The cells were incubated with 200 µg/ml Doxil® for 24 hours, washed, and 0.1% P85 was added for 1 hour. After 60 minutes incubation with Pluronic there was little if any colocalization with DOX from Doxil®. 24 hours later some co-localization was observed, however there was no nuclear uptake of the drug. Next the incubation with Doxil® was extended to 48 hours, pre- and post-treatment of P85 with 2 hours and localization could be observed in Fig. 3C.

Fluorescence quenching analysis of DOX fluorescence in Doxil® with and without 0.1% P85

DOX exists in a crystalline state inside the Doxil® liposome and therefore its fluorescence is quenched (by ca. 82%). Upon the release of the drug the fluorescence emission is increased. The fluorescence intensity of Doxil® was measured in the presence and absence of 0.1% P85 to evaluate the release of DOX from the liposomes. Fluorescence emission of Doxil® alone was not changed over the time period of 1 hour. Upon addition of Pluronic®, drastic increase of fluorescence was seen after 30 minutes of incubation and did not change during the following 30 minutes indicating that the release was fast and reached its maximum during first 30 minutes of exposure to Pluronic® (Fig. 4).

The effect of Pluronic® on doxorubicin release from DOXIL® *in vitro* was also determined. As seen in Figure 5, doxorubicin was released from the liposomes at higher rates in the presence of Pluronic® both at pH 5.5 and 7.4.

Anti-tumor efficacy of Doxil® and Pluronic P85 formulations

Antitumor effects of Doxil® in the presence or absence of Pluronic® formulation were evaluated to determine whether Pluronic® could enhance the drug accumulation in the tumor and lead to tumor suppression. A2780 ovarian tumor bearing mice received i.v. with 12 mg/kg single injection of Doxil®. One hour, 48 hours and 96 hours later, mice were received the same amount of 0.02% P85 after Doxil® administration. The tumor volumes were measured every other day. Tumor volume changes in control and treated groups are shown in Figure 6. Post treatment of Pluronic P85 induced significant tumor growth inhibition compared to the single dose of Doxil®, even in the 1 hour post treatment group. The anti-tumor efficacy was more pronounced in the 48 hours post treatment group. The observed difference in anti-tumor effects of Doxil® in the presence or absence of Pluronic® could be correlated to the pharmacokinetic characteristic of Doxil®, which is well known as the long blood circulation of DOX. As it was demonstrated above (Fig. 4), Pluronic® induces the increase of mobility and permeability of liposomal lipid bilayer, which attribute to the rapid release of the drug from liposomes.

To evaluate the systemic toxicity of different treatments heart, liver, spleen and kidneys were isolated from tumor bearing animals at the end point of the experiment. The histological analysis (Figure 7) did not show any toxicity in all cases.

A number of publications and patent documents are cited throughout the foregoing specification in order to describe the state of the art to which this invention pertains. The entire disclosure of each of these citations is incorporated by reference herein.

While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present invention, as set forth in the following claims.

What is claimed is:

1. A method of inhibiting a cancer in a subject, said method comprising administering at least one encapsulated chemotherapeutic agent and at least one amphiphilic block copolymer to said subject.

2. The method of claim 1, wherein said encapsulated chemotherapeutic agent and said amphiphilic block copolymer are contained within a single composition.

3. The method of claim 1, wherein said encapsulated chemotherapeutic agent and said amphiphilic block copolymer are contained within separate compositions.

4. The method of claim 3, wherein said encapsulated chemotherapeutic agent and said amphiphilic block copolymer are administered at least sequentially.

5. The method of claim 4, wherein said encapsulated chemotherapeutic agent is administered before said amphiphilic block copolymer.

6. The method of claim 1, wherein said encapsulated chemotherapeutic agent and said amphiphilic block copolymer are administered at least simultaneously.

7. The method of claim 1, wherein said encapsulated chemotherapeutic agent is a liposomal doxorubicin.

8. The method of claim 1, wherein said amphiphilic block copolymer comprises at least one block of ethylene oxide and at least one block of propylene oxide.

9. The method of claim 8, wherein said amphiphilic block copolymer is a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer.

10. The method of claim 9, wherein the poly(propylene oxide) content of the amphiphilic block copolymer is at least 30%.

11. The method of claim 9, wherein the molecular mass of the poly(propylene oxide) block is from about 1200 to about 4200.

12. The method of claim 1, comprising the administration of a first amphiphilic block copolymer and a second amphiphilic block copolymer, wherein the first and second amphiphilic block copolymers are different.

13. A composition comprising at least one encapsulated chemotherapeutic agent, at least one amphiphilic block copolymer, and at least one pharmaceutical carrier.

14. The composition of claim 13, wherein said encapsulated chemotherapeutic agent is a liposomal doxorubicin.

15. The composition of claim 13, wherein said amphiphilic block copolymer comprises at least one block of ethylene oxide and at least one block of propylene oxide.

16. A kit comprising:

a) a first composition comprising at least one encapsulated chemotherapeutic agent and at least one pharmaceutical composition; and

b) a second composition comprising at least one amphiphilic block copolymer and at least one pharmaceutical composition.

17. The kit of claim 16, wherein said encapsulated chemotherapeutic agent is a liposomal doxorubicin.

18. The kit of claim 16, wherein said amphiphilic block copolymer comprises at least one block of ethylene oxide and at least one block of propylene oxide.

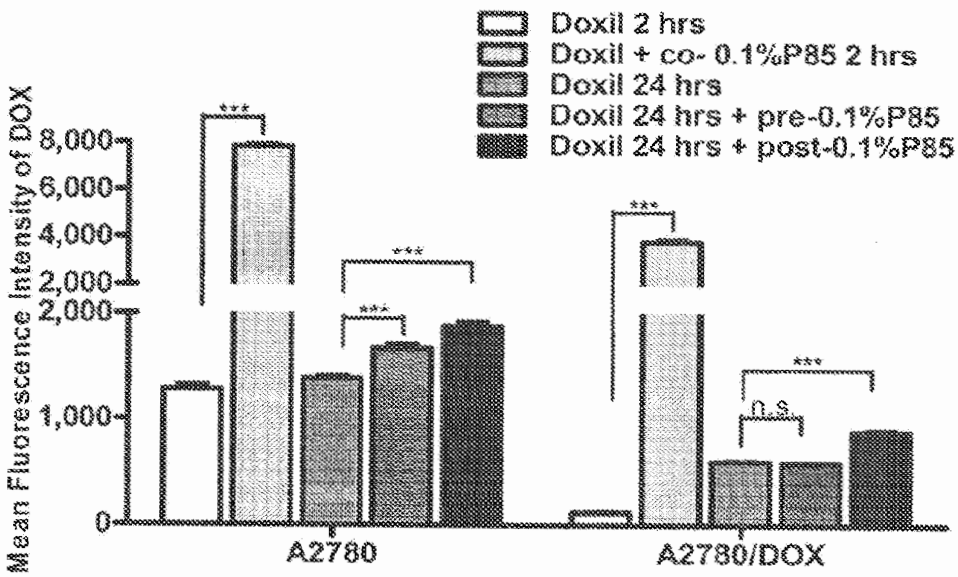

Doxil 2 hrs
Doxil + co- 0.1%P85 2 hrs
Doxil 24 hrs
Doxil 24 hrs + pre-0.1%P85
Doxil 24 hrs + post-0.1%P85
Mean Fluorescence Intensity of DOX
8,000
6,000
4,000
2,000
2,000
1,000
0
***
***
***
***
***
n.s.
A2780
A2780/DOX

Figure 1A

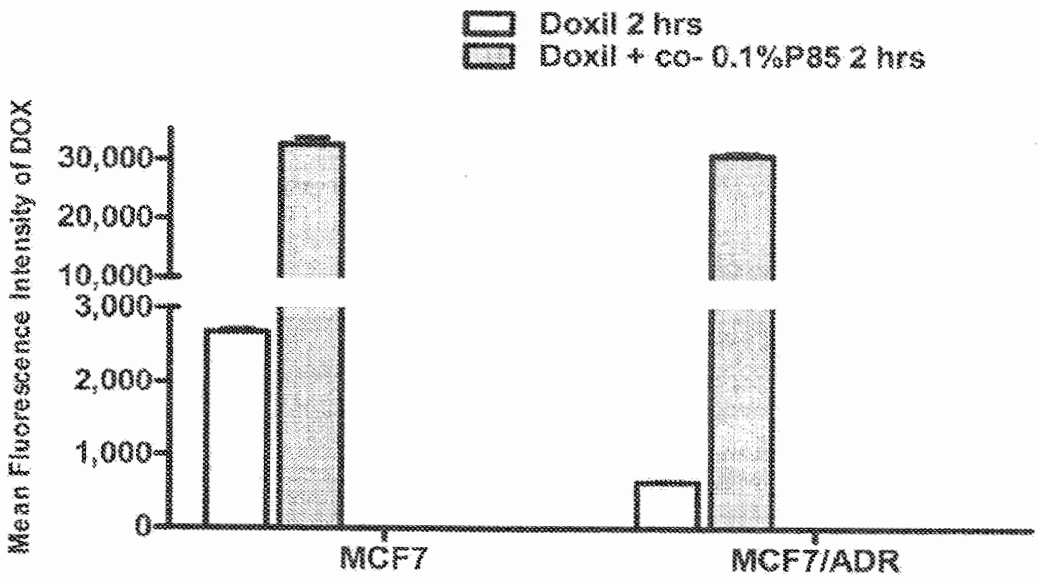

Doxil 2 hrs
Doxil + co- 0.1%P85 2 hrs
Mean Fluorescence Intensity of DOX
30,000
20,000
10,000
3,000
2,000
1,000
0
MCF7
MCF7/ADR

Figure 1B

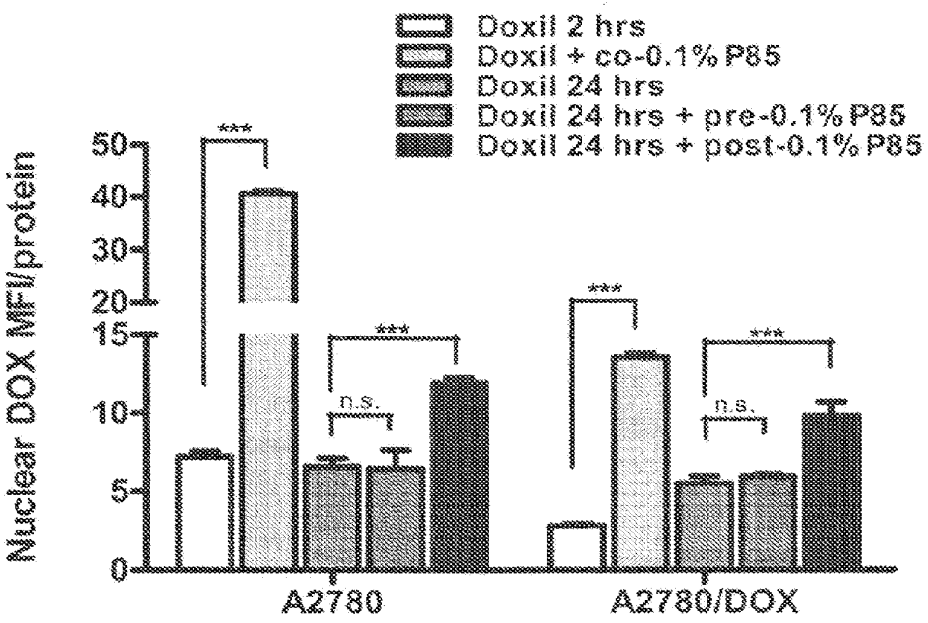

Doxil 2 hrs
Doxil + co-0.1% P85
Doxil 24 hrs
Doxil 24 hrs + pre-0.1% P85
Doxil 24 hrs + post-0.1% P85
Nuclear DOX MFI/protein
50
40
30
20
15
10
5
0
***
***
n.s.
***
***
n.s.
A2780
A2780/DOX

Figure 2

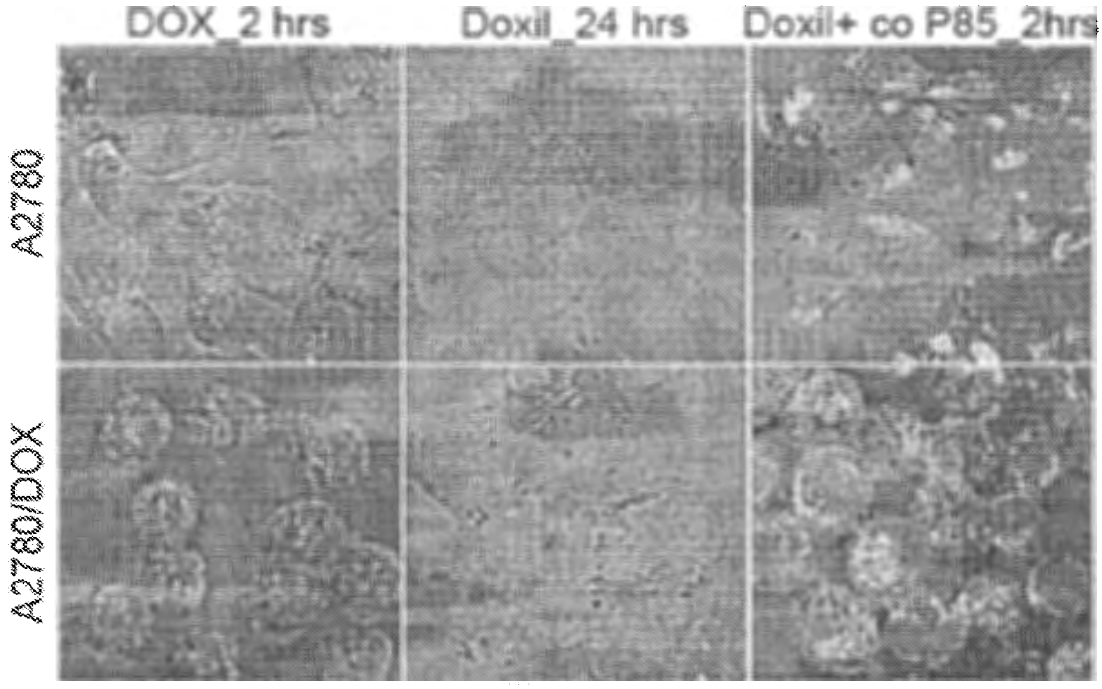


Figure 3A

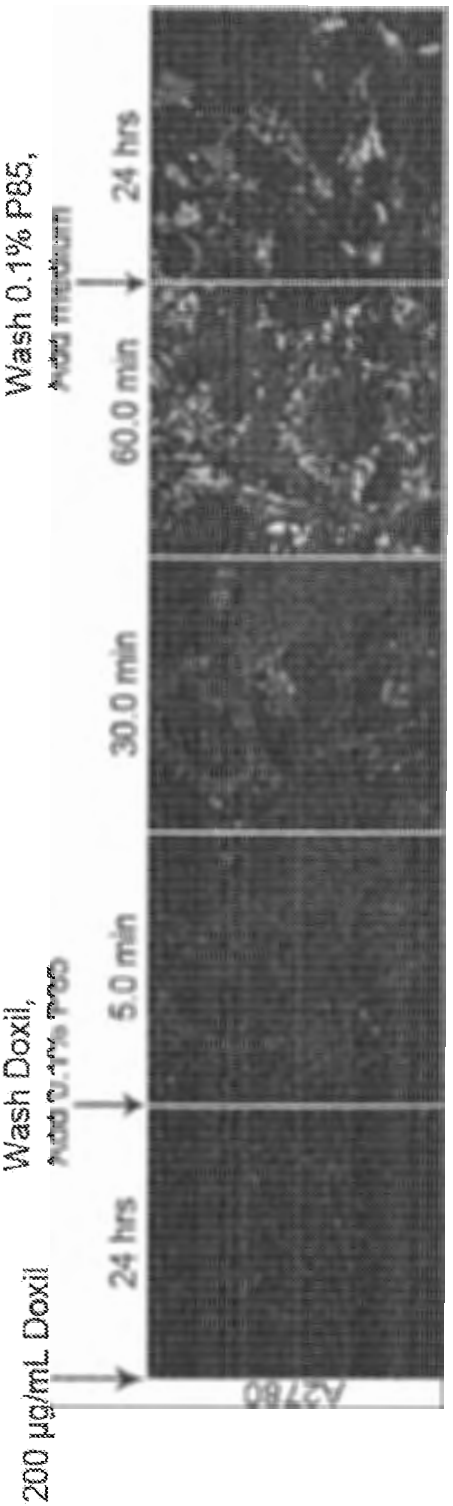

200 µg/mL Doxil
24 hrs
Wash Doxil,
5.0 min
30.0 min
60.0 min
Wash 0.1% P85,
24 hrs

Figure 3B

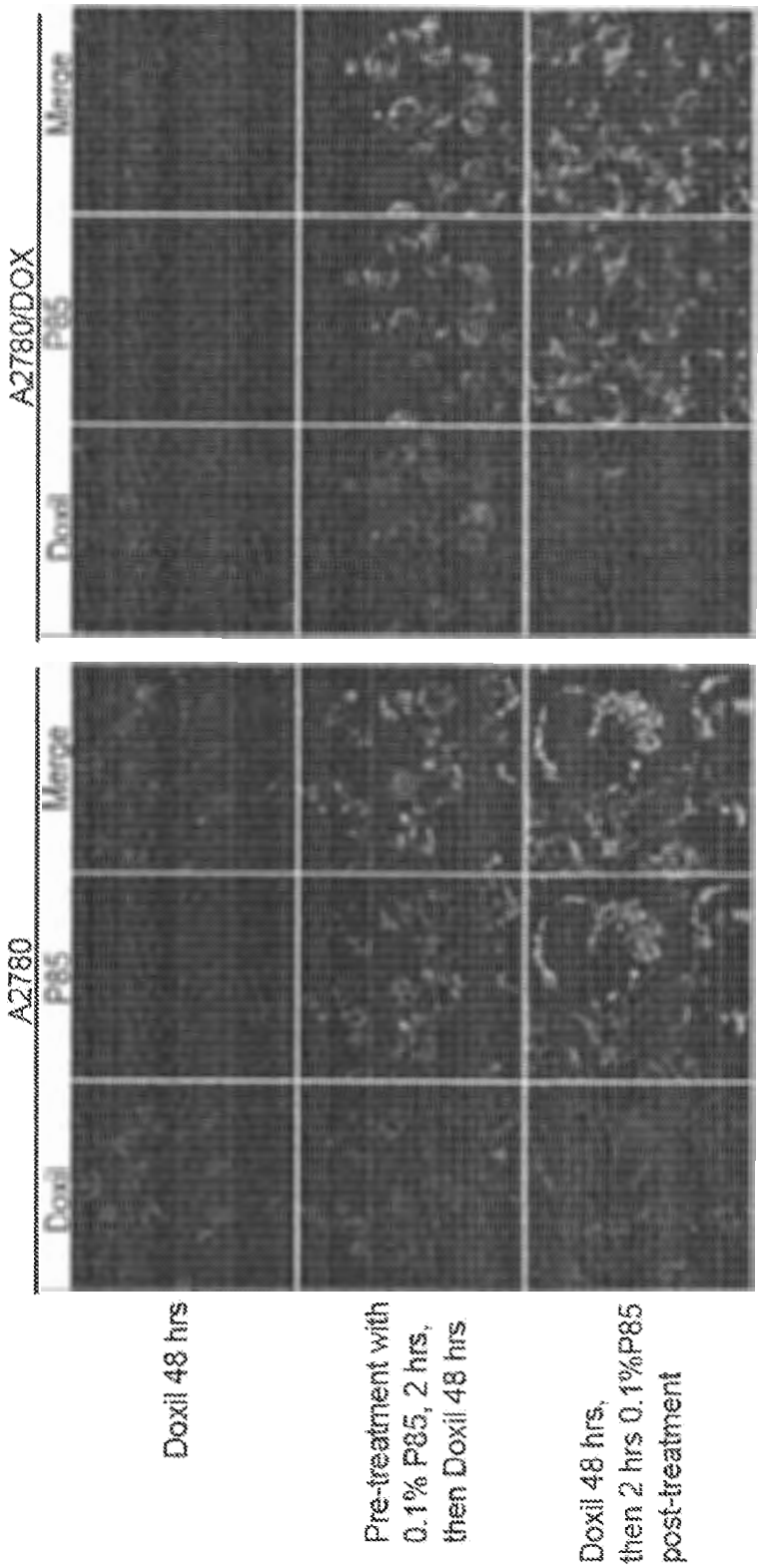
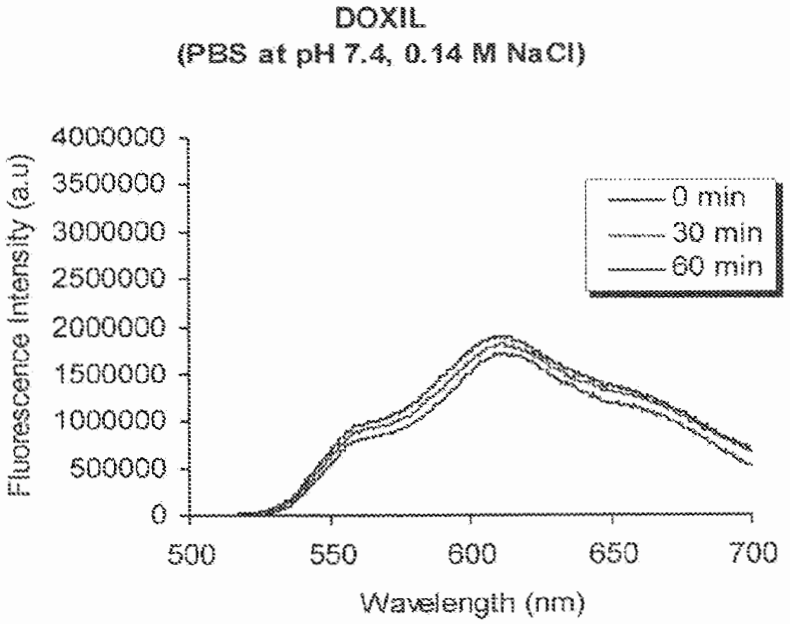

A2780
A2780/DOX
Doxil
P85
Merge
Doxil 48 hrs
Pre-treatment with 0.1% P85, 2 hrs, then Doxil 48 hrs
Doxil 48 hrs, then 2 hrs 0.1%P85 post-treatment

Figure 3C

A


DOXIL
(PBS at pH 7.4, 0.14 M NaCl)
Fluorescence Intensity (a.u)
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
500
550
600
650
700
Wavelength (nm)
0 min
30 min
60 min

B

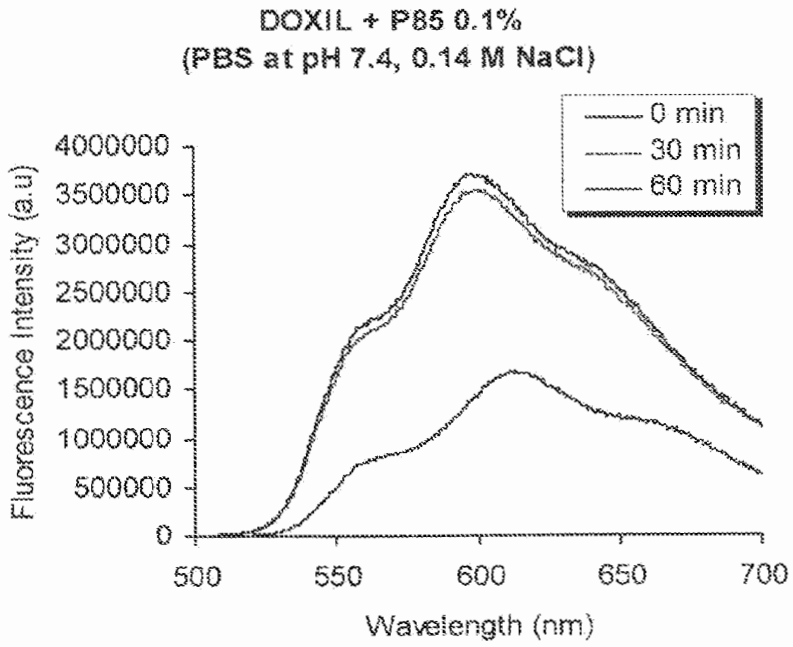

DOXIL + P85 0.1%
(PBS at pH 7.4, 0.14 M NaCl)
Fluorescence Intensity (a.u)
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
500
550
600
650
700
Wavelength (nm)
0 min
30 min
60 min

Figure 4

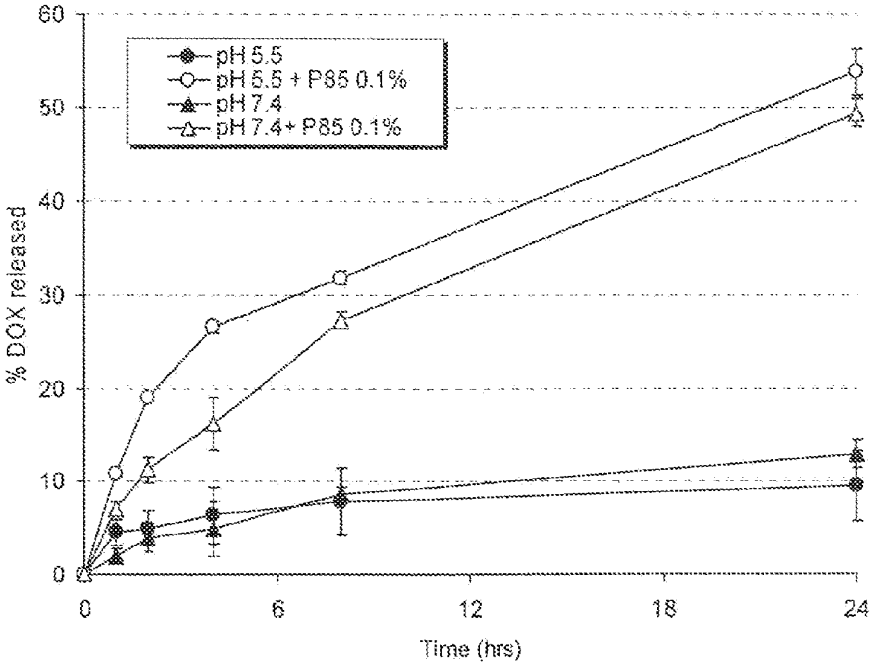

pH 5.5
pH 5.5 + P85 0.1%
pH 7.4
pH 7.4 + P85 0.1%
% DOX released
Time (hrs)
0
10
20
30
40
50
60
0
6
12
18
24

Figure 5

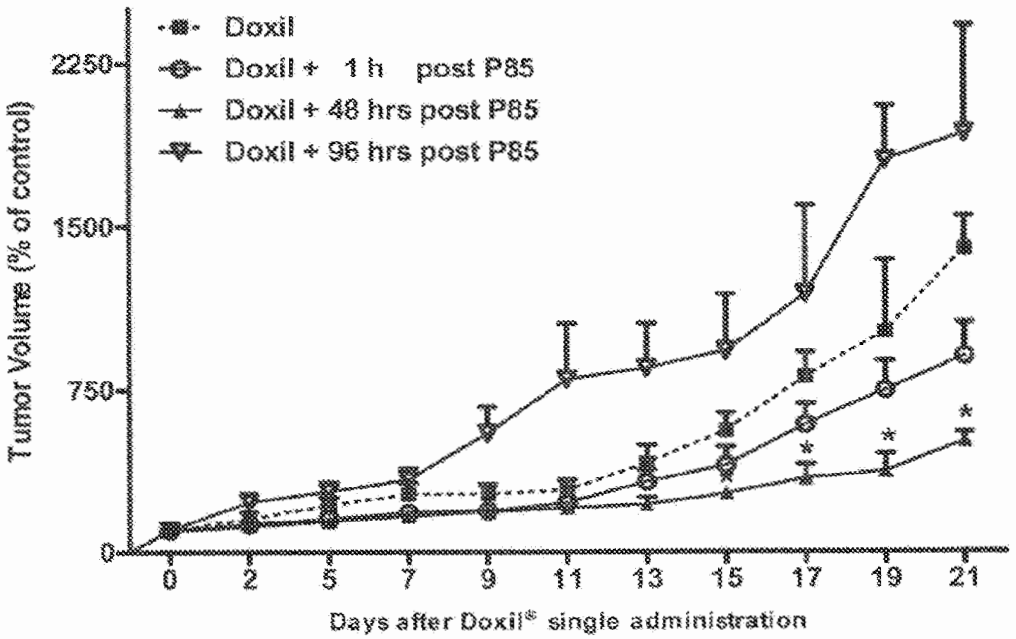

Doxil
Doxil + 1 h post P85
Doxil + 48 hrs post P85
Doxil + 96 hrs post P85
Tumor Volume (% of control)
2250
1500
750
0
0
2
5
7
9
11
13
15
17
19
21
Days after Doxil® single administration
*
*
*

Figure 6

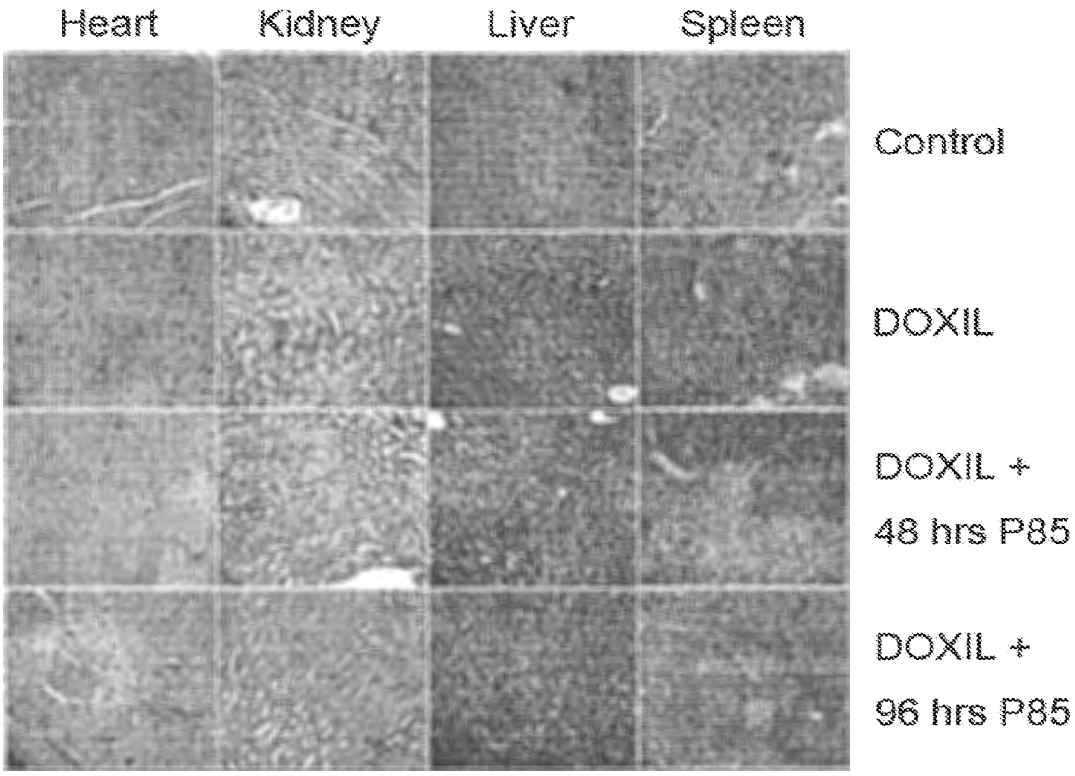

Heart
Kidney
Liver
Spleen
Control
DOXIL
DOXIL +
48 hrs P85
DOXIL +
96 hrs P85

Figure 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/50518

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 9/50, A61K 9/127 (2011.01)
USPC - 424/497
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)
USPC -- 424/497

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC -- 424/451, 424/450

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST -- PGPB,USPT,USOC,EPAB,JPAB; Dialog Classic Files -- 654, 652, 349, 348, 35, 65, 155; USPTO Web Page; Google Scholar -- encapsulated chemotherapeutic agent, doxorubicin, doxil, amphiphilic block copolymer, P85, triblock copolymer, simultaneous administration, sequential administration, PEO-PPO-PEO, cancer, kits

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2008/0075762 A1 (TARDI et al.) 27 March 2008 (27.03.2008) para [0027], [0028], [0074], [0077], [0078], [0080], [0081], [0086], [0096], [0127], [0130], [0140], [0154], [0155]	1-7, 13, 14, 16, 17 ---------- 8-12, 15, 18
Y	US 2008/0206187 A1 (EXNER et al.) 28 August 2008 (28.08.2008) para [0025], [0026], [0040], [0042], [0053], [0088]	8-12, 15, 18
X	US 2008/0181939 A1 (DISCHER et al.) 31 July 2008 (31.07.2008) para [0015], [0063], [0147], [0149], [0301], [0311], abstract	1, 3, 7, 16, 17

☐ 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
21 December 2011 (21.12.2011)	10 JAN 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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(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, BN, BR, BW, BY, BZ, CA, CH, CL, 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, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, 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, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2013/138371 A1

(54) **Title**: METHODS FOR TREATING PANCREATIC CANCER USING COMBINATION THERAPIES COMPRISING AN ANTI-ERBB3 ANTIBODY

(57) **Abstract**: Provided are methods for treating pancreatic cancer in a patient by co-administering combinations of an anti-ErbB3 antibody and one or more additional therapeutic agents. Further disclosed are the combinations of therapies which include: the an(i-ErbB3 antibody is coadministered with irinotecan, the anti-ErbB3 antibody is co-administered with paclitaxel (e.g., nab-paclitaxel), and the anti-ErbB3 antibody is coadministered with erlotinib and gemcitabine.

METHODS FOR TREATING PANCREATIC CANCER USING COMBINATION THERAPIES COMPRISING AN ANTI-ERBB3 ANTIBODY

Background

Despite improvements in cancer treatments, there remains a critical need to further improve therapies so as to prolong patients' lives while maintaining quality of life, particularly in the case of advanced cancers such as pancreatic cancers that often are, or become, resistant to current therapeutic modalities.

The ErbB3 receptor is 148 kD transmembrane receptor that belongs to the ErbB/EGFR receptor tyrosine kinase family; it is the only family member known to lack intrinsic kinase activity. The ErbB receptors form homo- and heterodimeric complexes with other ErbB receptors that impact the physiology of cells and organs by mediating ligand-dependent (or rarely ligand independent) activation of multiple signal transduction pathways. Upon binding one of its physiological ligands (*e.g.,* heregulin), ErbB3 heterodimerizes with another ErbB family member, typically ErbB2 (HER2). ErbB3/ErbB2 dimerization results in phosphorylation of ErbB3 on tyrosine residues of the intracellular cytoplasmic region of the protein. ErbB3-containing heterodimers in tumor cells have been shown to be the most mitogenic and oncogenic receptor complexes of ErbB family members, and they strongly activate intracellular signaling pathways involved in tumorigenesis, such as those promoting cell survival, growth, and migration.

Incidence of pancreatic cancer has markedly increased during the past several decades. It now ranks as the fourth leading cause of cancer death in the United States. Pancreatic cancer's high mortality rate is due to a dearth of effective therapies and a complete absence of reliably durable therapies. Because of the location of the pancreas, pancreatic cancer is typically not diagnosed until a tumor has become large enough to produce systemic symptoms. This, coupled with the absence of good screening tools and a limited understanding of risk factors, results in patients usually having advanced disease, often advanced metastatic disease, at the time of diagnosis. Metastatic pancreatic cancer has a dismal prognosis and is almost uniformly fatal, with an overall survival rate of less than 4% at 5 years.

There are few approved treatment options for advanced or metastatic pancreatic cancers, particularly for those of exocrine origin. Single-agent gemcitabine is the current standard of care in first-line treatment of advanced and metastatic pancreatic adenocarcinoma. In clinical trials, single-agent gemcitabine has consistently demonstrated a median prolongation of survival of 5 to 6 months and a 1-year survival rate of about 20%. Single agent gemcitabine was also approved as second line treatment for patients previously treated with but no longer responsive to 5-Fluorouracil, with a median overall prolongation of survival of 3.9 months.

Based upon what is known of the biology of pancreatic cancer, a variety of targeted agents have been evaluated, but only erlotinib, a protein tyrosine kinase inhibitor targeted to EGFR, has been approved for first-line use in advanced pancreatic cancer, and the approval is only for use in combination with gemcitabine. The co-administration of erlotinib with gemcitabine resulted in a statistically significant benefit in survival, and improvements in median survival (6.4 months vs. 5.9 months), and 1-year survival rate (24% vs. 17%) compared to gemcitabine alone. Clinical trials evaluating other targeted agents, including studies testing the antibodies bevacizumab and cetuximab, have been disappointingly negative. Thus, there is an urgent need for improvements in, and effective alternatives to, current therapies for pancreatic cancer. The disclosed invention addresses this need.

Summary

Monotherapy with an anti-ErbB3 antibody significantly suppresses tumor growth in a dose-dependent manner in *in vivo* pancreatic adenocarcinoma xenograft models. It has now been discovered that co-administration of an anti-ErbB3 antibody with one or more additional therapeutic agents, such as paclitaxel (*e.g.*, nab-paclitaxel), irinotecan, or erlotinib (with or without concomitant gemcitabine), exhibits therapeutic synergy.

Accordingly, provided are methods of treating pancreatic cancer in a patient by co-administering therapeutically synergistic combinations of an anti-ErbB3 antibody and one or more additional therapeutic agents. These methods include a method of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents, wherein the one of the one or more additional therapeutic agents comprise an EGFR inhibitor. In some embodiments the one or more additional therapeutic agents is an EGFR inhibitor that is selected from, gefitinib, erlotinib, afatinib and lapatinib. In some embodiments the one or more additional therapeutic agents is an EGFR inhibitor that is selected from MM-151, Sym004, cetuximab, panitumumab, zalutumumab, nimotuzumab, and matuzumab. In further embodiments the one or more additional therapeutic agents further comprise a nucleoside metabolic inhibitor (e.g., formulated for intravenous administration) such a gemcitabine and the EGFR inhibitor (e.g., formulated for oral administration) is optionally erlotinib.

Also provided are methods of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents wherein the one or more additional therapeutic agents comprise 1) a nucleoside metabolic inhibitor such as gemcitabine, or 2) a microtubule stabilizing agent (e.g., formulated for intravenous administration) such as paclitaxel injection, nab-paclitaxel and docetaxel. Such co-administrations beneficially have an additive or superadditive effect on suppressing pancreatic tumor

growth, which effect on suppressing pancreatic tumor growth is measured, *e.g.*, in a mouse xenograft model using BxPC-3 or COLO-357 cells.

Also provided are methods of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents wherein the one or more additional therapeutic agents comprise a topoisomerase 1 inhibitor and optionally wherein the topoisomerase 1 inhibitor is formulated for intravenous administration, such inhibitors are e.g., camptothecins selected from the group consisting of 9-aminocamptothecin, 7-ethylcamptothecin, 10-hydroxycamptothecin, 9-nitrocamptothecin, 10,11-methylenedioxycamptothecin, 9-amino-10,11-methylenedioxycamptothecin, 9-chloro-10,11-methylenedioxycamptothecin, topotecan, lurtotecan, silatecan, and irinotecan and when the camptothecin is irinotecan or topotecan the irinotecan or topotecan may be liposomally encapsulated irinotecan or liposomally encapsulated topotecan. When the one or more additional therapeutic agents is liposomally encapsulated irinotecan or liposomally encapsulated topotecan , the liposomally encapsulated irinotecan or liposomally encapsulated topotecan may each advantageously be contained in liposomes in the form of a sucrose octasulfate salt.

Also provided are methods of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents, wherein the one or more additional therapeutic agents is chosen from the group consisting of a bispecific anti-ErbB2/anti-ErbB3 antibody, an anti-IGF-1R/anti-ErbB3 antibody, an anti EGFR/anti-ErbB3 antibody, or a mixture of anti-EGFR and anti-ErbB3 antibodies.

Further provided are methods of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents, wherein i) the antibody is an antibody having the heavy chain sequence set forth in SEQ ID NO:2 and the light chain sequence set forth in SEQ ID NO:4, and ii) the one or more additional therapeutic agents comprises eribulin.

In each of the preceding methods the ErbB3 inhibitor may be an anti-ErbB3 antibody, *e.g.*, an anti-ErbB3 antibody comprising CDRH1, CDRH2, and CDRH3 sequences comprising the amino acid sequences set forth in SEQ ID NO: 5 (CDRH1) SEQ ID NO: 6 (CDRH2) and SEQ ID NO: 7 (CDRH3), and further comprises CDRL1, CDRL2, and CDRL3 sequences comprising the amino acid sequences set forth in SEQ ID NO: 8 (CDRL1) SEQ ID NO: 9 (CDRL2) and SEQ ID NO: 10 (CDRL3), or one comprising V_H and/or V_L regions comprising the amino acid sequences set forth in SEQ ID NOs: 2 and 4, respectively. Anti-ErbB3 antibodies may further be selected from GE-huMab-HER3, MEDI3379, 8B8 (ATCC HB-12070), 1B4C3, 2D1D12, AMG888 and AV-203.

Further provided is a composition for the treatment of pancreatic cancer, or for the manufacture of a medicament for the treatment of pancreatic cancer, in a patient, said treatment comprising co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents, wherein the one of the one or more additional therapeutic agents comprise 1) an orally available EGFR inhibitor, e.g., gefitinib, erlotinib, afatinib or lapatinib (frequently erlotinib), or a parenterally available EGFR inhibitor, e.g., MM-151, Sym004, cetuximab, panitumumab, zalutumumab, nimotuzumab, or matuzumab. In such combinations the one or more additional therapeutic agents may comprise a nucleoside metabolic inhibitor, e.g., gemcitabine; 2) a nucleoside metabolic inhibitor, e.g., gemcitabine; 3) a microtubule stabilizing agent, e.g., a taxane such as eribulin, paclitaxel injection, nab-paclitaxel or docetaxel (frequently nab-paclitaxel) – preferably co-administration of the anti-ErbB3 antibody and the taxane has an additive or superadditive effect on suppressing pancreatic tumor growth, as compared to administration of the anti-ErbB3 antibody alone or the taxane alone, wherein the effect on suppressing pancreatic tumor growth is measured in a mouse xenograft model using BxPC-3 or COLO-357 cells; 4) a topoisomerase 1 inhibitor, e.g., a camptothecin such as 9-aminocamptothecin, 7-ethylcamptothecin, 10-hydroxycamptothecin, 9-nitrocamptothecin, 10,11-methylenedioxycamptothecin, 9-amino-10,11-methylenedioxycamptothecin, 9-chloro-10,11-methylenedioxycamptothecin, lurtotecan, silatecan, or (frequently) topotecan or irinotecan, *e.g.*, liposomally encapsulated irinotecan or liposomally encapsulated topotecan, each encapsulated, *e.g.*, in the form of a sucrose octasulfate salt. In one embodiment, the combination treatments are useful for inhibiting the spread of cancer cells from the pancreas to other tissues.

In each of the preceding methods and compositions the co-administration of the anti-ErbB3 antibody and the additional chemotherapeutic agent or agents preferably has an additive or superadditive effect on suppressing pancreatic tumor growth, as compared to administration of the anti-ErbB3 antibody alone or the one or more additional chemotherapeutic agents alone, wherein the effect on suppressing pancreatic tumor growth is measured in a mouse xenograft model using BxPC-3 or COLO-357 cells. In certain embodiments of these methods, at least one of the one or more additional therapeutic agents is administered at a dosage that is a reduced dosage that provides less of the at least one additional therapeutic agent than is provided by a dosage recommended by the manufacturer of the at least one additional therapeutic agent for administration for the treatment of cancer in a patient who is not receiving concurrent anti-ErbB3 antibody therapy, *e.g.*, the reduced dosage is a dosage that is about half the dosage recommended by the manufacturer.

In each of the methods and compositions disclosed herein, the anti-ErbB3 antibody is advantageously formulated for intravenous administration. In each, the patient may have recurrent or persistent pancreatic cancer following primary chemotherapy and may have failed prior therapy with a

platinum-based therapeutic agent or have failed prior treatment with, or become resistant to treatment with one or more of a) a nucleoside analog therapeutic agent, b) a platinum-based therapeutic agent, c) a therapeutic agent, that is a topoisomerase 1 inhibitor and d) a therapeutic agent that is a tyrosine kinase inhibitor. In each of the additional therapeutic agent or agents may be administered following the administration of the anti-ErbB3 antibody; optionally, the topoisomerase 1 inhibitor may be administered before the administration of the anti-ErbB3 antibody or the topoisomerase 1 inhibitor and the anti-ErbB3 antibody are administered simultaneously. When a microtubule stabilizing agent is co-administered it may be administered before, after or concurrently with an anti-ErbB3 antibody. When EGFR inhibitor and nucleoside metabolic inhibitor are co-administered they may be administered before, after or concurrently with an anti-ErbB3 antibody.

In any of the foregoing methods and compositions the one or more additional therapeutic agents comprise two or more additional therapeutic agents, one of which is optionally gemcitabine. In the foregoing methods, the two or more additional therapeutic agents may be a combination of folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX), or a combination of folinic acid, 5-fluorouracil, and oxaliplatin (FOLFOX), or a combination of folinic acid, 5-fluorouracil, and irinotecan (FOLFIRI).

In any of the foregoing methods and compositions the anti-ErbB3 antibody may be formulated for intravenous administration and 1) is selected from the group comprising GE-huMab-HER3, MEDI3379, AMG888, AV-203, 8B8, 1B4C3 and 2D1D12, or 2) is selected from an antibody comprising V_H and/or V_L regions comprising the amino acid sequences set forth in SEQ ID NOs: 2 and 4, respectively, or 3) comprises CDRH1, CDRH2, and CDRH3 sequences comprising the amino acid sequences set forth in SEQ ID NO: 5 (CDRH1) SEQ ID NO: 6 (CDRH2) and SEQ ID NO: 7 (CDRH3), and further comprises CDRL1, CDRL2, and CDRL3 sequences comprising the amino acid sequences set forth in SEQ ID NO: 8 (CDRL1) SEQ ID NO: 9 (CDRL2) and SEQ ID NO: 10 (CDRL3).

Furthermore, in any of the foregoing methods the pancreatic cancer may be an exocrine pancreatic cancer selected from the group consisting of acinar cell carcinoma, adenocarcinoma, adenosquamous carcinoma, giant cell tumor, intraductal papillary-mucinous neoplasm (IPMN), mucinous cystadenocarcinoma, pancreatoblastoma, serous cystadenocarcinoma, and solid and pseudopapillary tumors; or an adenocarcinoma that is a pancreatic ductal carcinoma; or an endocrine pancreatic cancer selected from the group consisting of: Gastrinoma (Zollinger-Ellison Syndrome), Insulinoma, Nonfunctional Islet Cell Tumor, Somatostatinoma, and Vasoactive Intestinal Peptide-Releasing Tumor (VIPoma or Verner-Morrison Syndrome) – any of which may comprise a KRAS gene comprising a KRAS mutation such as KRAS G12S and may also or alternately comprise a BRAF mutation (e.g., BRAF V600E), in which case one of the one or more additional therapeutic agents is optionally a BRAF kinase inhibitor, frequently vemurafenib.

In any of the foregoing methods and compositions, the one or more additional therapeutic agents may comprise an mTOR inhibitor selected from the group consisting of temsirolimus, everolimus, sirolimus, and ridaforolimus, most commonly everolimus.

In any of the preceding methods and compositions, the treatment produces at least one therapeutic effect selected from the group consisting of: reduction of the rate of tumor growth, reduction in size of tumor, reduction of tumor mitotic index, reduction in number of metastatic lesions over time, complete response, partial response, stable disease, increase in overall response rate, or a pathologic complete response.

In any of the foregoing compositions, the ErbB3 inhibitor may be an anti-ErbB3 antibody, e.g., an anti-ErbB3 antibody comprising CDRH1, CDRH2, and CDRH3 sequences comprising VH and/or VL regions comprising the amino acid sequences set forth in SEQ ID NOs: 2 and 4, respectively, or comprising the amino acid sequences set forth in SEQ ID NO: 5 (CDRH1) SEQ ID NO: 6 (CDRH2) and SEQ ID NO: 7 (CDRH3), SEQ ID NO: 8 (CDRL1) SEQ ID NO: 9 (CDRL2) and SEQ ID NO: 10 (CDRL3). Alternately, the anti-ErbB3 antibody may be selected from 8B8, 1B4C3, 2D1D12, AMG888 and AV-203. Preferably co-administration of the anti-ErbB3 antibody and the additional chemotherapeutic agent or agents has an additive or superadditive effect on suppressing pancreatic tumor growth, as compared to administration of the anti-ErbB3 antibody alone or the one or more additional chemotherapeutic agents alone, wherein the effect on suppressing pancreatic tumor growth is measured in a mouse xenograft model using BxPC-3 or COLO-357 cells. In certain embodiments, at least one of the one or more additional therapeutic agents is administered at a dosage that is a reduced dosage that provides less of the at least one additional therapeutic agent than is provided by a dosage recommended by the manufacturer of the at least one additional therapeutic agent for administration for the treatment of pancreatic cancer in a patient who is not receiving concurrent anti-ErbB3 antibody therapy, optionally the reduced dose is a dose that is about half the dosage recommended by the manufacturer.

In any of the preceding methods and compositions, the effective amount optionally 1) achieves a synergistic effect in reducing tumor volume in the patient; or 2) achieves tumor stasis in the patient.

Brief Description of the Drawings

Figure 1 shows suboptimal and optimal doses of MM-121 (**Figure 1A**) and irinotecan hydrochloride (CPT-11) (**Figure 1B**) for inhibiting tumor growth in a BxPC3 xenograft model.

Figure 2 shows tumor regression in a BxPC3 xenograft model after treatment with either CPT-11 or MM-398 in combination with the suboptimal (150 µg Q3D – **Figure 2A**) or optimal (600µg Q3D – **Figure 2B**) dose of MM-121.

Figure 3 shows tumor growth inhibition in a COLO-357 xenograft model after treatment with varying doses of MM-121.

Figure 4 shows tumor growth inhibition in a COLO-357 xenograft model after treatment with a suboptimal dose of MM-121 (300 μg Q3D) in combination with nab-paclitaxel **(Figure 4A)** or the same dose of MM-121 in combination with nab-paclitaxel with and without gemcitabine **(Figure 4B)**.

Figure 5 shows tumor growth inhibition in a COLO-357 xenograft model after treatment with MM-121 at 300 μg Q3D, erlotinib, and gemcitabine, either alone or in two-way or three-way combinations **(Figure 5A)**. **Figures 5B-E** depict each distinct dose combination shown in Figure **5A**.

Figure 6 shows tumor growth inhibition in a pancreatic primary tumor explant model after three-way combination treatment with MM-121, gemcitabine, and erlotinib.

Figure 7 shows the effect of MM-121 in combination with nab-paclitaxel and MM-398 in bioluminescent orthotopic pancreatic model using luciferase-labeled BxPC3 cells (BxPC3-Luc-2).

Figure 8 is a graph showing the effect of MM-121 on tumor cell migration to the lung (Figure 8A) or the liver (Figure 8B) in a pancreatic cancer orthotopic model.

Detailed Description

Methods of combination therapy and combination compositions for treating pancreatic cancer in a patient are provided. In these methods, the cancer patient is treated with both an anti-ErbB3 antibody and one or more additional therapeutic agents selected, *e.g.*, from irinotecan, paclitaxel, nab-paclitaxel, erlotinib, gemcitabine, everolimus, and afatinib.

Definitions:

The terms "combination therapy," "co-administration," "co-administered" or "concurrent administration" (or minor variations of these terms) include simultaneous administration of at least two therapeutic agents to a patient or their sequential administration within a time period during which the first administered therapeutic agent is still present in the patient when the second administered therapeutic agent is administered.

The term "monotherapy" refers to administering a single drug to treat a disease or disorder in the absence of co-administration of any other therapeutic agent that is being administered to treat the same disease or disorder.

"Additional therapeutic agent" is used herein to indicate any drug that is useful for the treatment of a malignant pancreatic tumor other than a drug that inhibits heregulin binding to ErbB2/ErbB3 heterodimer.

"Antibody" describes a polypeptide comprising at least one antibody-derived antigen binding site (*e.g.*, VH/VL region or Fv, or complementarity determining region - CDR) that specifically binds to a specific antigen, *e.g.,* ErbB3. "Antibodies" include whole antibodies and any antigen binding fragment, *e.g.*, Fab or Fv, or a single chain fragment (*e.g.*, scFv), as well as bispecific antibodies and similar engineered variants, human antibodies, humanized antibodies, chimeric antibodies Fabs, Fab'2s, ScFvs, SMIPs, Affibodies®, nanobodies, or a domain antibodies, and may be of any of the following isotypes: IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgAsec, IgD, and IgE. The antibody may be a naturally occurring antibody or may be an antibody that has been altered (*e.g.*, by mutation, deletion, substitution, conjugation to a non-antibody moiety). For example, an antibody may include one or more variant amino acids (compared to a naturally occurring antibody) which change a property (*e.g.*, a functional property) of the antibody. For example, numerous such alterations are known in the art which affect, *e.g.*, half-life, effector function, and/or immune responses to the antibody in a patient. The term "antibody" thus includes whole antibodies and any antigen binding fragment (*i.e.*, "antigen-binding portion," *e.g.*, Fabs) or single chains thereof (*e.g.*, scFvs) as well as bispecific antibodies and similar engineered variants, provided that they retain the binding specificity of an antibody.

An "anti-ErbB3 antibody" is an antibody that immunospecifically binds to the ectodomain of ErbB3. Such binding to ErbB3 typically exhibits a binding affinity equal or greater than that indicated by a K_d of 50 nM (*i.e.*, a binding affinity corresponding to a K_d value of 50 nM, or a higher binding affinity as indicated by a lower K_d value such as 50 pM), *e.g.*, as measured by a surface plasmon resonance assay or a cell binding assay.

The terms "ErbB2," "HER2," and "HER2 receptor," as used interchangeably herein, refer to the protein product of the human neu oncogene, also referred to as the ErbB2 oncogene or the HER2 oncogene.

"Dosage" refers to parameters for administering a drug in defined quantities per unit time (*e.g.*, per hour, per day, per week, per month, etc.) to a patient. Such parameters include, *e.g.*, the size of each dose. Such parameters also include the configuration of each dose, which may be administered as one or more units, *e.g.*, taken at a single administration, *e.g.,* orally (*e.g.*, as one, two, three or more pills, capsules, etc.) or injected (*e.g.*, as a bolus). Dosage sizes may also relate to doses that are administered continuously (*e.g.*, as an intravenous infusion over a period of minutes or hours). Such parameters further include frequency of administration of separate doses, which frequency may change over time.

"Dose" refers to an amount of a drug given in a single administration.

"Effective treatment" refers to treatment producing a beneficial outcome, *e.g.*, amelioration of at least one symptom of a disease or disorder. A beneficial outcome can take the form of an improvement over baseline, which is generally an improvement over measurements, observations, or reported

symptoms made, *e.g.*, prior to, simultaneously with, or immediately following initiation of therapy. A beneficial outcome can also take the form of arresting, slowing, retarding, or stabilizing the progression of disease, *e.g.*, as indicated by changes in a biomarker. Effective treatment may also refer to improvement or alleviation of one or more symptoms of pancreatic cancer; *e.g.*, such treatment may reduce pain, increase patient mobility, reduce tumor size and/or number, increase longevity, reduce the rate of development of metastatic lesions, slow or reverse tumor growth, prevent or delay tumor recurrence, or inhibit, retard, slow or stop cancer cell infiltration into organs or tissues outside the pancreas.

"Effective amount" refers to an amount (administered in one or more doses) of an antibody, protein or additional therapeutic agent, which amount is sufficient to provide effective treatment.

The term "platinum-based therapeutic agent" refers to organoplatinum compounds (or treatment therewith), including for example oxaliplatin, carboplatin and cisplatin.

The disclosures if the following subsections should not be construed as limiting.

I. Anti-ErbB3 Antibody: An anti-ErbB3 antibody (*e.g.*, MM-121) is to be administered to a patient in a disclosed combination. MM-121 is a fully human anti-ErbB3 antibody currently undergoing Phase II clinical trials. MM-121 (also referred to as "Ab #6") and related human anti-ErbB3 antibodies are described in detail in U.S. patent No. 7,846,440, U.S. Patent Publication Nos. US 20100056761, and US 20100266584, and PCT Publication No. WO 2008/100624. Other anti-ErbB3 antibodies that may be used in a disclosed combination include any of the other anti-ErbB3 antibodies described in US patent No. 7,846,440, such as Ab #3 (SEQ ID NOs:14-21), Ab #14 (SEQ ID NOs:22-29), Ab #17 (SEQ ID NOs:30-37) or Ab #19 (SEQ ID NOs:38-45) or an antibody that competes with Ab #3, Ab #14, Ab #17 or Ab #19 for binding to ErbB3Additional examples of anti-ErbB3 antibodies that may be administered in accordance with the methods disclosed herein include antibodies disclosed in US patents and patent publications Nos. 7,285,649, 8,362,215, and 20100255010, as well as antibodies 1B4C3 (cat # sc-23865, Santa Cruz Biotechnology) and 2D1D12 (U3 Pharma AG), both of which are described in, *e.g.*, US Publication No. 20040197332 and are produced by hybridoma cell lines DSM ACC 2527 or DSM ACC 2517 (deposited at DSMZ) anti-ErbB3 antibodies disclosed in U.S. Patent No. 7,705,130 including but not limited to the anti-ErbB3 antibody referred to as AMG888 (U3-1287 -- U3 Pharma AG and Amgen), described in, *e.g.,* U.S. patent No. 7,705,130; the anti-ErbB3 antibody referred to as AV-203 (Aveo Pharmaceuticals) which is described in US patent publication No. 20110256154, and the monoclonal antibodies (including humanized versions thereof), such as 8B8 (ATCC® HB-12070™), described in U.S. patent No. 5,968,511. Additional examples include MEDI3379 (Medimmune), and GE-huMab-HER3 (Genentech), which is a glycoengineered anti-ErbB3 antibody. Other such examples include anti-ErbB3 antibodies that are multi-specific antibodies and comprise at least one anti-ErbB3 antibody (*e.g.*, one of the aforementioned anti-ErbB3 antibodies) linked to at least a second therapeutic antibody or to an

additional therapeutic agent. Examples of such antibodies include MM-141 and MM-111, described, e.g., in copending U.S. patent publication No. US 2011-0059076. Other suitable anti-ErbB3 antibodies also include pan-HER antibody compositions such as those disclosed, e.g., in PCT publication No. WO/2012/059857 (Symphogen) which describes antibody compositions targeting multiple ErbB family receptors. Yet other suitable anti-ErbB3 antibodies comprise either**: 1)** variable heavy (VH) and/or variable light (VL) regions encoded by the nucleic acid sequences set forth in SEQ ID NOs:1 and 3, respectively**, or 2)** VH and/or VL regions comprising the amino acid sequences set forth in SEQ ID NOs: 2 and 4, respectively**, or 3)** CDRH1, CDRH2, and CDRH3 sequences comprising the amino acid sequences set forth in SEQ ID NO: 5 (CDRH1) SEQ ID NO: 6 (CDRH2) and SEQ ID NO: 7 (CDRH3), and/or CDRL1, CDRL2, and CDRL3 sequences comprising the amino acid sequences set forth in SEQ ID NO: 8 (CDRL1) SEQ ID NO: 9 (CDRL2) and SEQ ID NO: 10 (CDRL3) as well as an antibody that binds to human ErbB3 and has at least 90% variable region sequence identity with the above-mentioned antibodies 1), 2), or 3). In one embodiment, the antibody has heavy and light chains comprising the amino acid sequences set forth in SEQ ID NOs 12 and 13, respectively. In another embodiment, the antibody competes for binding with and/or binds to the same epitope on human ErbB3 as any one of the above-mentioned antibodies. When the antibody is MM-121, the epitope typically comprises residues 92-104 of human ErbB3 (SEQ ID NO: 11). In other embodiments, the antibody is a fully human monoclonal antibody that binds to ErbB3 and, in living cells and either a) inhibits ErbB2/ErbB3 complex formation or b) prevents intracellular phosphorylation of ErbB3induced by any of the forms of each of the following: heregulin, EGF, TGFα, betacellulin, heparin-binding epidermal growth factor, biregulin, epigen, epiregulin, and amphiregulin, or does both a) and b).

Anti-ErbB3 antibodies described above, can be generated, *e.g.*, in prokaryotic or eukaryotic cells, using methods well known in the art, *e.g.*, in a cell line capable of glycosylating proteins, such as CHO cells.

II. Additional therapeutic agents:

Chemotherapy with one or more of 5-fluorouracil (5-FU) and gemcitabine has been shown to prolong survival in advanced pancreatic cancer. Many novel small molecules are being widely and actively researched as chemotherapeutic agents. These compounds include fluoropyrimidines, nucleoside analogues, platinum-based therapeutic agents, topoisomerase 1 inhibitors, antimicrotubule agents, BRAF inhibitors, proteasome inhibitors, vitamin D analogues, folinic acid (leucovorin or levoleucovorin), arachidonic acid pathway inhibitors, histone deacytylase inhibitors, farnesyltransferase inhibitors and epidermal growth factor receptor tyrosine kinase inhibitors. A combination therapy including folinic acid, 5-fluorouracil, and irinotecan (FOLFIRI), folinic acid, 5-fluorouracil, irinotecan and oxaliplatin

(FOLFIRINOX), or, less commonly, a combination of folinic acid, 5-fluorouracil, and oxaliplatin (FOLFOX) are also used to treat pancreatic cancer.

Additional therapeutic agents suitable for combination with anti-ErbB3 antibodies may further include: 1) EGFR inhibitors including but not limited to monoclonal antibody EGFR inhibitors (*e.g.* MM-151, Sym004, cetuximab, panitumumab, zalutumumab, nimotuzumab, and matuzumab), small molecule tyrosine kinase inhibitors (*e.g.*, afatinib, gefitinib, erlotinib, PKI-166, PD-158780, EKB-569, Tyrphostin AG 1478), dual inhibitors of EGFR and ErbB2 (*e.g.* afatinib and lapatinib), and pan-HER kinase inhibitors (*e.g.* CI-1033 (PD 183805), AC480, HM781-36B, AZD8931 and PF299804); 2) pyrimidine antimetabolites, *e.g.* the nucleoside metabolic inhibitor gemcitabine; 3) topoisomerase 1 inhibitors(*e.g.* irinotecan); 4) microtubule stabilizing agents (*e.g.* laulimalide, epothilone A, epothilone B, discodermolide, eleutherobin, sarcodictyin A, sarcodictyin B, cabazitaxel, paclitaxel, nab-paclitaxel or docetaxel); 5) BRAF inhibitors, (*e.g.* vemurafenib); 6) IGF1R inhibitors (e.g. dalotuzumab, XL228, BMS-754807 AMG-479, R1507, figitumumab, IMC-A12, and MM-141, a bispecific ErbB3/IGF1R inhibitor (further described in Lugovskoy *et al.*, copending commonly assigned U.S. Patent Application Serial No. 61/558,192, filed 11/10/2011, and PCT application No. PCT/US2012/034244) and molecule IGF1R inhibitors include XL228 and BMS-754807); 7) phosphoinositide-3-kinase (PI3K) inhibitors (e.g. CAL101 and PX-866); 8) mitogen activated kinase kinase (MEK) inhibitors (e.g. XL518, CI-1040, PD035901, selumetinib, and GSK1120212); and 9) mTOR inhibitors (*e.g.* everolimus, temsirolimus, sirolimus, or ridaforolimus). mTOR (mammalian target of rapamycin) is a serine/threonine protein kinase that regulates cell growth, proliferation, motility, survival, and protein synthesis and transcription. Rapamycin is now known as sirolimus, an mTOR inhibitor used as an immunosuppressant.

In certain combination therapy methods, one or more of the following therapeutic agents is co-administered to the patient with an anti-ErbB3 antibody.

Gemcitabine (Gemzar®) is indicated as first line therapy for pancreatic adenocarcinoma and is also used in various combinations to treat ovarian, breast and non-small-cell lung cancers. Gemcitabine HCl is 2′-deoxy-2′,2′-difluorocytidine monohydrochloride (-isomer) (MW=299.66) and is administered parenterally, typically by i.v. infusion.

Irinotecan (Camptosar®) (irinotecan hydrochloride injection), also referred to as CPT-11, is administered parenterally, typically by i.v. infusion. CPT-11 is approved in the United States for treatment of metastatic colon or renal cancer. CPT-11 is also used to treat colorectal, gastric, lung, uterine cervical and ovarian cancers.

In one embodiment, CPT-11 is administered in a stable nanoliposomal formulation, *e.g.*, the formulation referred to herein as "MM-398" (also known as PEP02). MM-398 may be provided as a sterile, injectable parenteral liquid for intravenous injection. MM-398 may be administered, for example,

at a dosage of 120mg/m^2. The required amount of MM-398 may be diluted, *e.g.*, in 500mL of 5% dextrose injection USP and infused over a 90 minute period.

An MM-398 liposome is a unilamellar lipid bilayer vesicle of approximately 80-140 nm in diameter that encapsulates an aqueous space which contains irinotecan complexed in a gelated or precipitated state as a salt with sucrose octasulfate. The lipid membrane of the liposome is composed of phosphatidylcholine, cholesterol, and a polyethyleneglycol-derivatized phosphatidyl-ethanolamine in the amount of approximately one polyethyleneglycol (PEG) molecule for 200 phospholipid molecules. MM-398 recently achieved primary efficacy endpoints in Phase II clinical trials in metastatic pancreatic cancer and in gastric cancer, and is being investigated in the context of metastatic colorectal cancer.

Paclitaxel is administered parenterally, typically by i.v. infusion, and is formulated with polyethoxylated castor oil as "Taxol® (paclitaxel) injection" or with human serum albumin as "Abraxane® (paclitaxel protein-bound particles for injectable suspension) (albumin bound)" also called nab-paclitaxel. Paclitaxel is used to treat, *e.g.*, breast cancer, non-small cell lung cancer (in combination with cisplatin), and AIDS-related Kaposi's sarcoma.

Erlotinib (Tarceva®) is orally administered and is used to treat, *e.g.*, locally advanced or metastatic non-small cell lung cancer (NSCLC) and locally advanced, unresectable or metastatic pancreatic cancer (in combination with gemcitabine).

Afatinib (Tomtovok®) is an orally administered tyrosine kinase inhibitor that irreversibly inhibits HER2 and EGFR kinases. It is not yet marketed and is being tested in the context of non-small cell lung carcinoma, breast, prostate, head and neck cancers, and glioma.

Temsirolimus (Torisel®) is an mTOR inhibitor that is administered parenterally, typically by i.v. infusion and is used to treat advanced renal cell carcinoma.

Everolimus (Afinitor®), a 40-O-(2-hydroxyethyl) derivative of sirolimus, is an mTOR inhibitor that is administered orally and is used to treat progressive neuroendocrine tumors of pancreatic origin (PNET) in patients with unresectable, locally advanced or metastatic disease.

Vemurafenib (Zelboraf®) is a BRAF enzyme inhibitor approved for the treatment of late-stage melanoma in patients whose cancer harbors a V600E BRAF mutation.

III. Combination Therapies

As herein provided, anti-ErbB3 antibodies (*e.g.,* MM-121) are co-administered with one or more additional therapeutic agents (*e.g.* irinotecan, nab-paclitaxel, erlotinib, gemcitabine, everolimus, and/or afatinib), to provide effective treatment to human patients having a pancreatic cancer (*e.g.*, pancreatic adenocarcinoma).

The anti-ErbB3 antibody and one or more additional therapeutic agents for combination therapy may be administered to the patient in any suitable form. Typically, each of the anti-ErbB3 antibody and

the one or more additional therapeutic agents is provided in the form of a pharmaceutical composition, which comprises the antibody or additional therapeutic agent in a physiologically acceptable carrier. In certain embodiments, the one or more additional therapeutic agents are formulated for oral or intravenous administration. In another embodiment, the anti-ErbB3 antibody is formulated for intravenous administration.

In particular embodiments, the anti-ErbB3 antibody is administered at a dose selected from: 2-50 mg/kg (body weight of the patient) administered once a week, or twice a week or once every three days, or once every two weeks, and 1-100 mg/kg administered once a week, or twice a week or once every three days, or once every two weeks. In various embodiments, the anti-ErbB3 antibody is administered at a dosage of 3.2 mg/kg, 6 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg or 40 mg/kg at a timing of once a week, or twice a week or once every three days, or once every two weeks. Additional dosage ranges for the anti-ErbB3 antibody include: 1-1000 mg/kg, 1-500 mg/kg, 1-400 mg/kg, 1-300 mg/kg and 1-200 mg/kg. Suitable dosage schedules include once every three days, once every five days, once every seven days (*i.e.*, once a week), once every 10 days, once every 14 days (*i.e.*, once every two weeks), once every 21 days (*i.e.*, once every three weeks), once every 28 days (*i.e.*, once every four weeks) and once a month.

IV. Patient Populations

In one embodiment, a human patient for treatment using the methods and compositions disclosed herein exhibits evidence of recurrent or persistent disease following primary chemotherapy.

In another embodiment, such a human patient has had and failed at least one prior platinum based chemotherapy regimen for management of primary or recurrent disease, *e.g.*, a chemotherapy regimen comprising carboplatin, cisplatin, or another organoplatinum compound.

In an additional embodiment, the human patient has failed prior treatment with gemcitabine or become resistant to gemcitabine.

As used herein the terms "resistant" and "refractory" refer to tumor cells that survive treatment with a therapeutic agent. Such cells may have responded to a therapeutic agent initially, but subsequently exhibited a reduction of responsiveness during treatment, or did not exhibit an adequate response to the therapeutic agent in that the cells continued to proliferate in the course of treatment with the agent. In one embodiment a resistant or refractory tumor is one where the treatment-free interval following completion of a course of therapy for a patient having the tumor is less than 6 months (*e.g.*, owing to recurrence of the cancer) or where there is tumor progression during the course of therapy.

In another embodiment, the pancreatic cancer undergoing treatment is advanced pancreatic cancer, which is a pancreatic tumor that exhibits either or both of distant metastasis or peripancreatic extension of the tumor.

The combination therapies and methods disclosed herein are useful for the treatment of pancreatic cancers, including pancreatic cancers that are refractory or resistant to other anti-cancer treatments. The methods can be used in the treatment of essentially any type of pancreatic cancer tumor that expresses ErbB3. Examples of types of pancreatic cancers to be treated include 1) exocrine pancreatic cancers, *e.g.,* acinar cell carcinoma, adenocarcinoma, adenosquamous carcinoma, giant cell carcinoma of the pancreas, intraductal papillary-mucinous neoplasm (IPMN), mucinous cystadenocarcinoma, pancreatoblastoma, and serous cystadenocarcinoma, and 2) endocrine pancreatic cancers, *e.g.,* gastrinoma (Zollinger-Ellison Syndrome), insulinoma, nonfunctional islet cell tumor, somatostatinoma, vasoactive intestinal peptide-releasing tumor (VIPoma or Verner-Morrison Syndrome). In one embodiment, the pancreatic cancer is an adenocarcinoma (*i.e.*, pancreatic ductal carcinoma).

In one embodiment, the pancreatic cancer comprises one or more KRAS mutations (*e.g.,* a KRAS G12S mutation). "KRAS mutation" refers to oncogenic mutations found in certain cancers in KRAS, the human homolog of the v-Ki-ras2 Kirsten rat sarcoma viral oncogene. It has been reported that KRAS mutations are found in 73% of pancreatic tumors. In another embodiment, the pancreatic cancer comprises a BRAF mutation (*e.g.,* a BRAF V600E mutation). "BRAF mutation" refers to oncogenic mutations in the BRAF (Serine/threonine-protein kinase B-Raf or "B-Raf") gene. When present, KRAS and BRAF mutations are typically found together in pancreatic tumors. Transgenomic, Inc., Omaha, Nebraska; Asuragen, Inc., Austin, Texas; EntroGen, Inc., Tarzana, California; and QIAGEN Gmbh, Hilden, Germany, are among the many companies that market both KRAS and BRAF testing kits. Multiple laboratories now offer KRAS and BRAF mutation testing of tumor biopsy samples as a commercial service. *e.g.,* GenPath, Elmwood Park, New Jersey and Clarient, Inc., Aliso Viejo, California.

V. Outcomes

As shown in the Examples herein, co-administration of an anti-ErbB3 antibody with one or more additional therapeutic agents (*e.g.* irinotecan, nab-paclitaxel, erlotinib, gemcitabine, everolimus, and/or afatinib) provides improved efficacy compared to treatment with the antibody alone or with the one or more additional therapeutic agents in the absence of antibody therapy. Preferably, a combination of an anti-ErbB3 antibody with one or more additional therapeutic agents exhibits therapeutic synergy.

"Therapeutic synergy" refers to a phenomenon where treatment of patients with a combination of therapeutic agents manifests a therapeutically superior outcome to the outcome achieved by each individual constituent of the combination used at its optimum dose (T. H. Corbett et al., 1982, Cancer Treatment Reports, 66, 1187). In this context a therapeutically superior outcome is one in which the patients either a) exhibit fewer incidences of adverse events while receiving a therapeutic benefit that is equal to or greater than that where individual constituents of the combination are each administered as monotherapy at the same dose as in the combination, or b) do not exhibit dose-limiting toxicities while

receiving a therapeutic benefit that is greater than that of treatment with each individual constituent of the combination when each constituent is administered in at the same doses in the combination(s) as is administered as individual components. In xenograft models, a combination, used at its maximum tolerated dose, in which each of the constituents will be present at a dose generally not exceeding its individual maximum tolerated dose, manifests therapeutic synergy when decrease in tumor growth achieved by administration of the combination is greater than the value of the decrease in tumor growth of the best constituent when the constituent is administered alone.

Thus, in combination, the components of such combinations have an additive or superadditive effect on suppressing pancreatic tumor growth, as compared to monotherapy with the anti-ErbB3 antibody or treatment with the chemotherapeutic(s) in the absence of antibody therapy. By "additive" is meant a result that is greater in extent (e.g., in the degree of reduction of tumor mitotic index or of tumor growth or in the degree of tumor shrinkage or the frequency and/or duration of symptom-free or symptom-reduced periods) than the best separate result achieved by monotherapy with each individual component, while "superadditive" is used to indicate a result that exceeds in extent the sum of such separate results. In one embodiment, the additive effect is measured as slowing or stopping of pancreatic tumor growth. The additive effect can also be measured as, e.g., reduction in size of a pancreatic tumor, reduction of tumor mitotic index, reduction in number of metastatic lesions over time, increase in overall response rate, or increase in median or overall survival.

One non-limiting example of a measure by which effectiveness of a therapeutic treatment can be quantified is by calculating the log10 cell kill, which is determined according to the following equation:

$$\log10 \text{ cell kill} = T\,C \text{ (days)}/3.32 \times Td$$

in which T C represents the delay in growth of the cells, which is the average time, in days, for the tumors of the treated group (T) and the tumors of the control group (C) to have reached a predetermined value (1 g, or 10 mL, for example), and Td represents the time, in days necessary for the volume of the tumor to double in the control animals. When applying this measure, a product is considered to be active if log10 cell kill is greater than or equal to 0.7 and a product is considered to be very active if log10 cell kill is greater than 2.8. Using this measure, a combination, used at its own maximum tolerated dose, in which each of the constituents is present at a dose generally less than or equal to its maximum tolerated dose, exhibits therapeutic synergy when the log10 cell kill is greater than the value of the log10 cell kill of the best constituent when it is administered alone. In an exemplary case, the log10 cell kill of the combination exceeds the value of the log10 cell kill of the best constituent of the combination by at least 0.1 log cell kill, at least 0.5 log cell kill, or at least 1.0 log cell kill.

VI. Kits and Unit Dosage Forms

Kits that include a pharmaceutical composition containing an anti-ErbB3 antibody, such as MM-121, and a pharmaceutically-acceptable carrier, in a therapeutically effective amount adapted for use in the preceding methods are provided. The kits can optionally also include instructions, *e.g.*, comprising administration schedules, to allow a practitioner (*e.g.*, a physician, nurse, or patient) to administer the compositions contained therein to a patient having a pancreatic cancer. In one embodiment, the kit further comprises irinotecan. In another embodiment, the kit further comprises paclitaxel (*e.g.*, nab-paclitaxel). In another embodiment, the kit further comprises erlotinib and/or gemcitabine. In another embodiment the kit includes infusion devices such as needles, catheters, tubing, and the like. Optionally, the kits include multiple packages each containing a single dose amount of the antibody or of the chemotherapeutic (*e.g.*, in a unit dosage form distributed by the manufacturer) for administration in accordance with the methods provided herein.

VII. Treatment of Cancer Types other than Pancreatic Cancer

ErbB3 is a critical activator of phosphoinositide 3-kinase (PI3K) signaling in cancers that arise from dependence on the epidermal growth factor receptor, *e.g.*, pancreatic cancer, and reactivation of ErbB3 is a prominent method by which a cancer can become resistant to ErbB inhibitors. The methods and combination treatments described herein will thus be useful for treatment of types of cancer with a molecular pathology similar to that of pancreatic cancer in that they are EGFR-driven and, after anti-EGFR treatment, become resistant to such treatment through increased signaling in the PI3K pathway via ErbB3. Such cancer types include, but are not limited to, lung, colon, head and neck, and esophageal cancers.

The following examples are illustrative and should not be construed as limiting the scope of this disclosure in any way; many variations and equivalents will become apparent to those skilled in the art upon reading the present disclosure.

Incorporation By Reference: The disclosure of each and every US, International, or other patent or patent application or publication referred to herein is hereby incorporated herein by reference in its entirety.

Examples

Example 1 Combination Treatment with MM-121 and irinotecan Inhibits Tumor Growth in Pancreatic Cancer

The anti-tumor efficacy and tolerability of MM-121 and irinotecan (CPT-11 or in liposomal formulation (MM-398)), either alone (*i.e.*, as a monotherapy) or in combination, in tumor-bearing mice was evaluated using human pancreatic adenocarcinoma BxPC-3 cells (ATCC # CRL-1687) implanted as xenografts in nu/nu nude mice. BxPC-3 cells were derived from a human metastatic tumor and expressed

high levels of HRG and EGFR. In these xenograft studies, nu/nu nude mice were obtained from Charles River Laboratories International. The mice were housed in Tecniplast® Individually Ventilated polycarbonate (Makrolon®) Cages (IVC) set in climate-controlled rooms and had free access to food and acidified water. 8×10^6 cells were mixed 1:1 in reduced growth factor Matrigel™ (BD Biosciences, Cat # 354230) and implanted by subcutaneous injection into the left flank of female, 4-5 week old nu/nu mice. Tumors were allowed to reach 150 mm^3 in size before randomization.

Dose Escalation Study A dose escalation study was performed to determine suboptimal and optimal doses of MM-121 and CPT-11 in preparation for combination therapy using the BxPC-3 xenograft model.

Xenograft-bearing mice were randomized into 10 groups of 5 mice, containing mice with a similar size distribution of tumors. Four groups were treated with escalating intraperitoneal (i.p.) doses of MM-121 (75, 150, 300 or 600 µg, Q3D per group), 3 groups were treated with escalating doses of irinotecan (CPT-11) (6.25, 12.5, 25 or 50 mg/kg, Q7D, per group), one control group was treated with PBS, Q3D, and another control group was treated with 5% DMSO in PBS (CPT-11 vehicle), Q7D. Treatment continued for 3 weeks. Tumors were measured twice weekly, and tumor volume was calculated as $\pi/6 \times \text{length} \times \text{width}^2$, where the width is the shorter measurement.

Dose responses for inhibition of tumor growth were observed for MM-121 (Figure 1A) and CPT-11 (Figure 1B). The "suboptimal" doses for evaluation in combination therapy in BxPC-3 xenografts were identified as 150 µg Q3D for MM-121 and 12.5 to 25 mg/kg for CPT-11 Q7D. Meanwhile, the "optimal" doses for evaluation in BxPC-3 xenografts were identified as 600µg Q3D for MM-121 and 50 mg/kg for CPT-11.

Combination therapy study A combination therapy study was performed to demonstrate the effects of various combinations of MM-121, irinotecan (CPT-11), and liposomal irinotecan (MM-398).

Mice were randomized as above into 9 groups of 5 mice each. Five groups were treated with i.p. doses of a single agent alone, as follows: (1) MM-121 (150 µg Q3D), (2) MM-121 (600 µg Q3D), (3) CPT-11 (25 mg/kg Q7D), (4) MM-398 (10 mg/kg Q3D), or (5) PBS (Q3D) alone (Control). Four groups were treated with a combination therapy of (1) MM-121 and CPT-11 or (2) MM-121 and MM-398 with the doses described above. Treatment continued for 4 weeks. Tumors were measured twice weekly and tumor volume calculated.

As shown in Figure 2A (MM-121 dose; 150 µg Q3D) and Figure 2B (MM-121 optimal dose; 600µg Q3D), MM-121 as a single agent significantly suppressed tumor growth in a dose-dependent manner. Moreover, while CPT-11 and MM-398 alone each inhibited tumor growth *in vivo*, combination treatments with MM-121 and CPT-11 or MM-121 and MM-398 exhibited an additive effect on tumor growth inhibition, as compared to tumor growth inhibition observed with each of the individual agents.

Furthermore, treatment with either CPT-11 or MM-398 in combination with the optimal dose of MM-121 (600μg Q3D) resulted in pronounced tumor regression.

Example 2 Combination Treatment with MM-121 and nab-paclitaxel Inhibits Tumor Growth in Pancreatic Cancer

The anti-tumor efficacy and tolerability of MM-121 and nab-paclitaxel (paclitaxel protein-bound particles for injectable suspension) combination treatment, with or without gemcitabine, was evaluated using COLO-357 cells (ECACC Cat # 94072245) implanted as xenografts in nu/nu nude mice. COLO-357 cells were derived from a lymph node metastasis of a human non-endocrine pancreatic cancer, and have been reported to harbor KRAS G12S and BRAF V600E mutations.

Xenograft-bearing mice were prepared as described except 5 x 10^6 COLO-357 cells were used.

Dose Escalation Study – MM-121 A dose escalation study was performed to determine optimal doses of MM-121 in preparation for combination therapy using the COLO-357 xenograft model.

Xenograft-bearing mice were randomized into seven groups of five mice, containing mice with a similar size distribution of tumors. Six groups were treated with escalating doses of i.p. MM-121 (18.75, 37.5, 75, 150, 300 or 600 μg, Q3D per group), and another control group was treated with PBS. Treatment continued for 4 weeks. Tumors were measured twice weekly, and tumor volume was calculated. As depicted in Figure 3, MM-121 suppressed tumor growth in a dose-dependent manner. The "suboptimal" doses for evaluation in combination therapy in COLO-357 cells xenografts were identified as 300 μg Q3D for MM-121, and the "optimal" dose was identified as 600μg Q3D.

Combination therapy study A combination therapy study was performed to demonstrate the effects of various combinations of MM-121, nab-paclitaxel (Abraxis; Catalog # NDC68817-134-50), and gemcitabine (LC labs; Catalog # G1477).

Xenograft-bearing mice were randomized into 10 groups of 9-10 mice, containing mice with a similar size distribution of tumors. Four groups were treated with i.p. doses of a single agent alone, as follows: (1) 10 mg/kg Q3D i.p. of nab-paclitaxel, (2) 20 mg/kg Q3D i.p. of nab-paclitaxel (3) 300 μg Q3D i.p. of MM-121, (4) 150mg/kg Q7D i.p. of gemcitabine. Three groups were treated with duo combination therapy, as follows: (1) MM-121 and nab-paclitaxel (10 mg/kg), (2) MM-121 and nab-paclitaxel (20 mg/kg), and (3) MM-121 and gemcitabine (150mg/kg). Two groups were treated with triple combination therapy, as follows: (1) MM-121, nab-paclitaxel (10 mg/kg) and gemcitabine, and (2) MM-121, nab-paclitaxel (20 mg/kg) and gemcitabine. A control group was treated with PBS, Q3D, i.p.. Treatment continued for 7 weeks. Tumors were measured twice weekly, and tumor volume was calculated.

As shown in Figures 4A-B, MM-121 as a single agent, at the suboptimal dose of 300 μg Q3D, significantly suppressed tumor growth in a dose-dependent manner. However, the COL-357 xenograft

model responded poorly to nab-paclitaxel alone (Figure 4A) and moderately to gemcitabine alone (Figure 4B) for the doses tested.

With respect to the combination therapies, MM-121 in combination with nab-paclitaxel showed a dose-dependent additive effect on tumor growth suppression when compared to each drug alone (Figure 4A). Additionally, MM-121 in combination with gemcitabine shows little if any enhancement over MM-121 single therapy (Figure 4B) in COLO-357 tumors.

Moreover, while CPT-11 and MM-398 alone each inhibit tumor growth *in vivo*, treatment with the combinations of MM-121 and CPT-11 or MM-121 and MM-398 resulted in an additive effect on suppression of tumor growth, as compared to treatment with each of the individual agents. Furthermore, treatment with either CPT-11 or MM-398 in combination with the optimal dose of MM-121 (600µg Q3D) resulted in pronounced increase in cell death, as shown in figure 4.

The triple combination therapy of MM-121, nab-paclitaxel and gemcitabine suppressed tumor growth to a similar extent as the dual MM-121 and nab-paclitaxel combination, indicating that gemcitabine did not enhance the inhibitory effect of this dual combination (Figure 4B) in COLO-357 tumors.

Example 3 Triple Combination Treatment with MM-121, gemcitabine, and erlotinib Inhibits Tumor Growth in Pancreatic Cancer

A) A combination therapy study was performed to demonstrate the effects of various combinations of MM-121, erlotinib (LC Laboratories – Catalog # E4007), and gemcitabine. Specifically, the anti-tumor efficacy of MM-121 and erlotinib in combination, with or without gemcitabine, on tumor-bearing mice was analyzed using COLO-357 cells. Xenograft bearing mice were prepared as described in the preceding Example.

Five groups were treated with single agents, as follows: (1) 50 mg/kg Q5D oral of erlotinib, (2) 100 mg/kg Q5D oral of erlotinib, (3) 300 µg Q3D i.p. of MM-121, (4) 150 mg/kg Q7D i.p. of gemcitabine, and (5) 300 mg/kg Q7D i.p. of gemcitabine. Additional groups were treated with double combination or triple combination therapy, as set forth in Table 1 below. A control group was treated with PBS, Q3D, i.p. Treatment continued for 7 weeks. Tumors were measured twice weekly and tumor volume was calculated.

Table 1

Group	Test Article	Dose	Route	Dose Schedule	Dose Volume	Mice (n)
1	Vehicle Control	NA	IP	Q3D X 12	0.1 ml	10
2	MM-121	300 µg	IP	Q3D X 12	0.1 ml	10

3	erlotinib	50 mg/kg	PO	QD (5x a week)	0.2 ml	10
4	erlotinib	100 mg/kg	PO	QD (5x a week)	0.2 ml	10
5	gemcitabine	150 mg/kg	IP	Q7D X 5	0.4ml	10
6	gemcitabine	300 mg/kg	IP	Q7D X 5	0.4ml	10
7	MM121 + erlotinib + gemcitabine	300µg+50mpk+150mpk	IP+PO+IP	Q3D X 12+ QD (5x a week)+ Q7D X 5	0.1 ml+0.2 ml+0.4ml	10
8	MM121 + erlotinib + gemcitabine	300µg+50mpk+300mpk	IP+PO+IP	Q3D X 12+ QD (5x a week)+ Q7D X 5	0.1 ml+0.2 ml+0.4ml	10
9	MM121 + erlotinib + gemcitabine	300µg+100mpk+150mpk	IP+PO+IP	Q3D X 12+ QD (5x a week)+ Q7D X 5	0.1 ml+0.2 ml+0.4ml	10
10	MM121 + erlotinib + gemcitabine	300µg+100mpk+150mpk	IP+PO+IP	Q3D X 12+ QD (5x a week)+ Q7D X 5	0.1 ml+0.2 ml+0.4ml	10
11	erlotinib + gemcitabine	50 mpk+150 mpk	PO+IP	QD (5x a week)+ Q7D X 5	0.2 ml+0.4ml	10
12	erlotinib + gemcitabine	100 mpk+300 mpk	PO+IP	QD (5x a week)+ Q7D X 5	0.2 ml+0.4ml	10
13	erlotinib + gemcitabine	50 mpk+300 mpk	PO+IP	QD (5x a week)+ Q7D X 5	0.2 ml+0.4ml	10
14	erlotinib + gemcitabine	100 mpk+150 mpk	PO+IP	QD (5x a week)+ Q7D X 5	0.2 ml+0.4ml	10

IP: interperitoneal administration - PO: oral administration

As shown in Figures 5A-E, the triple combination of MM-121, erlotinib and gemcitabine was superior in inhibiting tumor growth compared to all doses tested of the standard of care therapy (*i.e.*,

gemcitabine and erlotinib). These results indicate that the addition of MM-121 to the standard of care regimen is beneficial for tumor growth control.

B) To further demonstrate the effects of various combinations of MM-121, erlotinib and gemcitabine on pancreatic cancer, , a pancreatic primary tumor explant model (*i.e.*, low passage Champions Tumorgrafts™ CTG-0289 (PANC002), which is reported to harbor KRAS mutations) was used. Immunocompromised mice (Harlan®; nu/nu) between 4-6 weeks of age were implanted unilaterally on the right flank with tumor fragments harvested from 2-4 host animals each implanted from a specific passage lot. Pre-study tumor volumes were recorded for each experiment beginning approximately one week prior to its estimated start date. When tumors reach approximately 125-225 mm^3, animals were matched by tumor volume into treatment and control groups and dosing initiated (Day 0), as set forth in Table 2. Animals in all studies were tagged and followed individually throughout the experiment.

Beginning Day 0, tumor dimensions were measured twice weekly by digital caliper, and data including individual and mean estimated tumor volumes (Mean TV ± SEM) were recorded for each group. Tumor volume was calculated using the formula (1): $TV = width^2 \times length \times 0.52$. At study completion, percent tumor growth inhibition (%TGI) values was calculated and reported for each treatment group (T) versus control (C) using initial (i) and final (f) tumor measurements by the formula (2): $\%TGI = 1 - T_f - T_i \ / \ C_f - C_i$.

The results are set forth in Table 2 and Figure 6.

Table 2: *In Vivo* Evaluation of MM-121 in Pancreas Tumorgraft™ Model

Group	-n-	Agent	Dose (mg/kg/dose)	ROA/ Schedule*
1	9	Vehicle Control	--	i.p./ q3dx10
2	9	MM-121	30	i.p./ q3dx10
3	9	erlotinib	35	p.o./ qdx28
4	9	gemcitabine	60	i.p./ q3dx4
5	9	erlotinib	35	p.o./ qdx28
		gemcitabine	60	i.p./ q3dx4
6	9	MM-121	30	i.p./ q3dx10
		erlotinib	35	p.o./ qdx28
7	9	MM-121	30	i.p./ q3dx10
		gemcitabine	60	i.p./ q3dx4
8	9	MM-121	30	i.p./ q3dx10
		erlotinib	35	p.o./ qdx28
		gemcitabine	60	i.p./ q3dx4

*gemcitabine was dosed first and MM-121 was dosed second with administration occurring two hours apart from each other.

As shown in Figure 6, MM-121, gemcitabine or erlotinib treatment as single agents yielded suboptimal effects on tumor growth inhibition for the doses tested. Specifically, this pancreatic primary tumor explant model was moderately sensitive to gemcitabine and exhibited lesser responses to erlotinib or MM-121.

The combination of gemcitabine and erlotinib was no more efficacious than gemcitabine alone. Additionally, while MM-121 in combination with gemcitabine showed an additive effect as compared to the single agents, MM-121 in combination with erlotinib was not more efficacious than MM-121 alone in this model.

In contrast, the triple combination of MM-121, erlotinib, and gemcitabine had an additive effect on tumor growth inhibition, as compared to the agents alone or paired. In sum, the addition of MM-121 addition to the standard of care combination (erlotinib plus gemcitabine) in this primary pancreatic explant model provided enhanced tumor growth inhibition.

Example 4. Effect of MM-121 in combination with chemotherapies in an orthotopic model of pancreatic cancer

Luciferase-labeled human pancreatic cancer cells (BxPC-3-luc2 Bioware® Ultra, Caliper Life Sciences) were expanded in culture and inoculated orthotopically into nude mice (Charles River, nu/nu). Mice were anesthetized and a 0.5cm incision was made on the left flank region. The spleen and the tail of the pancreas were exteriorized. Cells were inoculated at $1x10^6$ cells/ 20µl into the sub-capsular space into the tail of the pancreas. The spleen and the pancreas were then placed back into the peritoneal cavity, and the cavity was sutured and skin closed with surgical staples.

In vivo whole body biophotonic imaging was performed weekly throughout the study. Seven days after inoculation of tumor cells the first bioluminescent imaging was performed and mice were randomized into 7 treatment groups (10 mice/group) and treated with PBS (Q3D, i.p.), MM121 600µg, 1200ug (Q3D, i.p.), MM398 10mg/kg (Q7D, i.v.), nab-paclitaxel 15mg/kg (Q3D, i.p.), or combination of MM121 600µg with either MM398 or nab-paclitaxel at the doses mentioned above.

Mice were treated via the regimen described above for 35 days; bioluminescent imaging was performed once every 7 days. At the end of the study, the mice were sacrificed 24 hours after the final dose of each treatment was administered, and final images were taken. The tumors were removed, imaged and placed in formalin for future evaluation. Selected organs such as lung, diaphragm, liver and gastrointestinal-associated lymph nodes were also removed at the end of study, placed in petri dishes and imaged for bioluminescence, as a measurement of tumor cell migration.

As shown in Figure 7, treatment with the combination of MM-121 and either MM-398 or nab-paclitaxel significantly decreased the tumor growth as compared to either drug treatment alone. In addition, MM-121 treatment significantly diminished tumor cell migration from pancreas to lung (Figure 8A) and liver (Figure 8B). Similar results were seen in the diaphragm and gastrointestinal-associated lymph nodes. Thus, these combination treatments are useful for inhibiting the spread of cancer cells from the pancreas to other organs.

SUMMARY OF SEQUENCES

MM-121 Heavy Chain Variable Region Nucleotide Sequence (SEQ ID NO:1)

gaggtgcagc tgctggagag cggcggaggg ctggtccagc caggcggcag cctgaggctg 60

tcctgcgccg ccagcggctt caccttcagc cactacgtga tggcctgggt gcggcaggcc 120

ccaggcaagg gcctggaatg ggtgtccagc atcagcagca gcggcggctg gaccctgtac 180

gccgacagcg tgaagggcag gttcaccatc agcagggaca acagcaagaa caccctgtac 240

ctgcagatga acagcctgag ggccgaggac accgccgtgt actactgcac caggggcctg 300

aagatggcca ccatcttcga ctactggggc cagggcaccc tggtgaccgt gagcagc 357

MM-121 VH amino acid sequence (SEQ ID NO:2)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYVMAWVRQAPGKGLEWVSSISSSGGWTLYADS
VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRGLKMATIFDYWGQGTLVTVSS

MM-121 Light Chain Nucleotide Sequence (SEQ ID NO:3)

cagtccgccc tgacccagcc cgccagcgtg agcggcagcc caggccagag catcaccatc 60

agctgcaccg gcaccagcag cgacgtgggc agctacaacg tggtgtcctg gtatcagcag 120

caccccggca aggcccccaa gctgatcatc tacgaggtgt cccagaggcc cagcggcgtg 180

agcaacaggt tcagcggcag caagagcggc aacaccgcca gcctgaccat cagcggcctg 240

cagaccgagg acgaggccga ctactactgc tgcagctacg ccggcagcag catcttcgtg 300

atcttcggcg agggaccaa ggtgaccgtc cta 333

MM-121 VL amino acid sequence (SEQ ID NO:4)
QSALTQPASVSGSPGQSITISCTGTSSDVGSYNVVSWYQQHPGKAPKLIIYEVSQRPSGVSNRFSG
SKSGNTASLTISGLQTEDEADYYCCSYAGSSIFVIFGGGTKVTVL

MM-121 VH CDR1 (SEQ ID NO:5)
HYVMA

MM-121 VH CDR2 (SEQ ID NO:6)
SISSSGGWTLYADSVKG

MM-121 VH CDR3 (SEQ ID NO:7)
GLKMATIFDY

MM-121 VL CDR1 (SEQ ID NO:8)
TGTSSDVGSYNVVS

MM-121 VL CDR2 (SEQ ID NO:9)
EVSQRPS

MM-121 VL CDR3 (SEQ ID NO:10)
CSYAGSSIFVI

ErbB3 (SEQ ID NO:11)
SEVGNSQAVCPGTLNGLSVTGDAENQYQTLYKLYERCEVVMGNLEIVLTGHNADLSFLQWIRE
VTGYVLVAMNEFSTLPLPNLRVVRGTQVYDGKFAIFVMLNYNTNSSHALRQLRLTQLTEILSGG
VYIEKNDKLCHMDTIDWRDIVRDRDAEIVVKDNGRSCPPCHEVCKGRCWGPGSEDCQTLTKTIC
APQCNGHCFGPNPNQCCHDECAGGCSGPQDTDCFACRHFNDSGACVPRCPQPLVYNKLTFQLEP
NPHTKYQYGGVCVASCPHNFVVDQTSCVRACPPDKMEVDKNGLKMCEPCGGLCPKACEGTGS
GSRFQTVDSSNIDGFVNCTKILGNLDFLITGLNGDPWHKIPALDPEKLNVFRTVREITGYLNIQSW
PPHMHNFSVFSNLTTIGGRSLYNRGFSLLIMKNLNVTSLGFRSLKEISAGRIYISANRQLCYHHSLN
WTKVLRGPTEERLDIKHNRPRRDCVAEGKVCDPLCSSGGCWGPGPGQCLSCRNYSRGGVCVTH
CNFLNGEPREFAHEAECFSCHPECQPMEGTATCNGSGSDTCAQCAHFRDGPHCVSSCPHGVLGA
KGPIYKYPDVQNECRPCHENCTQGCKGPELQDCLGQTLVLIGKTHLTMALTVIAGLVVIFMMLG
GTFLYWRGRRIQNKRAMRRYLERGESIEPLDPSEKANKVLARIFKETELRKLKVLGSGVFGTVH

KGVWIPEGESIKIPVCIKVIEDKSGRQSFQAVTDHMLAIGSLDHAHIVRLLGLCPGSSLQLVTQYL
PLGSLLDHVRQHRGALGPQLLLNWGVQIAKGMYYLEEHGMVHRNLAARNVLLKSPSQVQVAD
FGVADLLPPDDKQLLYSEAKTPIKWMALESIHFGKYTHQSDVWSYGVTVWELMTFGAEPYAGL
RLAEVPDLLEKGERLAQPQICTIDVYMVMVKCWMIDENIRPTFKELANEFTRMARDPPRYLVIK
RESGPGIAPGPEPHGLTNKKLEEVELEPELDLDLDLEAEEDNLATTTLGSALSLPVGTLNRPRGSQ
SLLSPSSGYMPMNQGNLGESCQESAVSGSSERCPRPVSLHPMPRGCLASESSEGHVTGSEAELQE
KVSMCRSRSRSRSPRPRGDSAYHSQRHSLLTPVTPLSPPGLEEEDVNGYVMPDTHLKGTPSSREG
TLSSVGLSSVLGTEEEDEDEEYEYMNRRRRHSPPHPPRPSSLEELGYEYMDVGSDLSASLGSTQS
CPLHPVPIMPTAGTTPDEDYEYMNRQRDGGGPGGDYAAMGACPASEQGYEEMRAFQGPGHQA
PHVHYARLKTLRSLEATDSAFDNPDYWHSRLFPKANAQRT

MM-121 Heavy Chain Amino Acid Sequence (SEQ ID NO:12)

1 EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYVMAWVRQA PGKGLEWVSS
51 ISSSGGWTLY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCTRGL
101 KMATIFDYWG QGTLVTVSSA STKGPSVFPL APCSRSTSES TAALGCLVKD
151 YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSNFGTQTY
201 TCNVDHKPSN TKVDKTVERK CCVECPPCPA PPVAGPSVFL FPPKPKDTLM
251 ISRTPEVTCV VVDVSHEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTFRV
301 VSVLTVVHQD WLNGKEYKCK VSNKGLPAPI EKTISKTKGQ PREPQVYTLP
351 PSREEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPMLDSDG
401 SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK

MM-121 Light Chain Amino Acid Sequence (SEQ ID NO:13)

1 QSALTQPASV SGSPGQSITI SCTGTSSDVG SYNVVSWYQQ HPGKAPKLII
51 YEVSQRPSGV SNRFSGSKSG NTASLTISGL QTEDEADYYC CSYAGSSIFV
101 IFGGGTKVTV LGQPKAAPSV TLFPPSSEEL QANKATLVCL VSDFYPGAVT
151 VAWKADGSPV KVGVETTKPS KQSNNKYAAS SYLSLTPEQW KSHRSYSCRV
201 THEGSTVEKT VAPAECS

Ab # 3 VH amino acid sequence (SEQ ID NO:14)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSAYNMRWVRQAPGKGLEWVSVIYPSGGATRYADS
VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGYYYYGMDVWGQGTLVTVSS

Ab # 3 VL amino acid sequence (SEQ ID NO:15)
QSVLTQPPSASGTPGQRVTISCSGSDSNIGRNYIYWYQQFPGTAPKLLIYRNNQRPSGVPDRISGS
KSGTSASLAISGLRSEDEAEYHCGTWDDSLSGPVFGGGTKLTVL

Ab # 3 VH CDR1 (SEQ ID NO:16)
AYNMR

Ab # 3 VH CDR2 (SEQ ID NO:17)
VIYPSGGATRYADSVKG

Ab # 3 VH CDR3 (SEQ ID NO:18
GYYYYGMDV

Ab # 3 VL CDR1 (SEQ ID NO:19)
SGSDSNIGRNYIY

Ab # 3 VL CDR2 (SEQ ID NO:20)
RNNQRPS

Ab # 3 VL CDR3 (SEQ ID NO:21)
GTWDDSLSGPV

Ab # 14 VH amino acid sequence (SEQ ID NO:22)
EVQLLESGGGLVQPGGSLRLSCAASGFTFSAYGMGWVRQAPGKGLEWVSYISPSGGHTKYADS
VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKVLETGLLVDAFDIWGQGTMVTVSS

Ab # 14 VL amino acid sequence (SEQ ID NO:23)
QYELTQPPSVSVYPGQTASITCSGDQLGSKFVSWYQQRPGQSPVLVMYKDKRRPSEIPERFSGSN
SGNTATLTISGTQAIDEADYYCQAWDSSTYVFGTGTKVTVL

Ab # 14 VH CDR1 (SEQ ID NO:24)
AYGMG

Ab # 14 VH CDR2 (SEQ ID NO:25)
YISPSGGHTKYADSVKG

Ab # 14 VH CDR3 (SEQ ID NO:26)
VLETGLLVDAFDI

Ab # 14 VL CDR1 (SEQ ID NO:27)
SGDQLGSKFVS

Ab # 14 VL CDR2 (SEQ ID NO:28)
YKDKRRPS

Ab # 14 VL CDR3 (SEQ ID NO:29)
QAWDSSTYV

Ab # 17 VH amino acid sequence (SEQ ID NO:30)
EVQLLESGGGLVQPGGSLRLSCAASGFTFSWYGMGWVRQAPGKGLEWVSYISPSGGITVYADS
VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARLNYYYGLDVWGQGTTVTVSS

Ab # 17 VL amino acid sequence (SEQ ID NO:31)
QDIQMTQSPSSLSASVGDRITITCQASQDIGDSLNWYQQKPGKAPRLLIYDASNLETGVPPRFSGS
GSGTDFTFTFRSLQPEDIATYFCQQSANAPFTFGPGTKVDIK

Ab # 17 VH CDR1 (SEQ ID NO:32)
WYGMG

Ab # 17 VH CDR2 (SEQ ID NO:33)
YISPSGGITVYADSVKG

Ab # 17 VH CDR3 (SEQ ID NO:34)
LNYYYGLDV

Ab # 17 VL CDR1 (SEQ ID NO:35)
QASQDIGDSLN

Ab # 17 VL CDR2 (SEQ ID NO:36)
DASNLET

Ab # 17 VL CDR3 (SEQ ID NO:37)
QQSANAPFT

Ab # 19 VH amino acid sequence (SEQ ID NO:38)
EVQLLESGGGLVQPGGSLRLSCAASGFTFSRYGMWWVRQAPGKGLEWVSYIGSSGGPTYYVDS
VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAGGRGTPYYFDSWGQGTLVTVSS

Ab # 19 VL amino acid sequence (SEQ ID NO:39)
QYELTQPASVSGSPGQSITISCTGTSSDIGRWNIVSWYQQHPGKAPKLMIYDVSNRPSGVSNRFSG
SKSGNTASLTISGLQAEDEADYYCSSYTSSSTWVFGGGTKLTVL

Ab # 19 VH CDR1 (SEQ ID NO:40)
RYGMW

Ab # 19 VH CDR2 (SEQ ID NO:41)
YIGSSGGPTYYVDSVKG

Ab # 19 VH CDR3 (SEQ ID NO:42)
GRGTPYYFDS

Ab # 19 VL CDR1 (SEQ ID NO:43)
TGTSSDIGRWNIVS

Ab # 19 VL CDR2 (SEQ ID NO:44)
DVSNRPS

Ab # 19 VL CDR3 (SEQ ID NO:45)
SSYTSSSTWV

What is claimed is:

1. A method of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents, wherein the one of the one or more additional therapeutic agents comprise an EGFR inhibitor.

2. The method of claim 1, wherein the one or more additional therapeutic agents is an EGFR inhibitor that is selected from, gefitinib, erlotinib, afatinib and lapatinib.

3. The method of claim 1, wherein the one or more additional therapeutic agents is an EGFR inhibitor that is selected from MM-151, Sym004, cetuximab, panitumumab, zalutumumab, nimotuzumab, and matuzumab.

4. The method of claim 1, wherein the one or more additional therapeutic agents further comprise a nucleoside metabolic inhibitor.

5. The method of claim 4, wherein the nucleoside metabolic inhibitor is gemcitabine.

6. The method of claim 5, wherein the EGFR inhibitor is erlotinib.

7. A method of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents wherein the one or more additional therapeutic agents comprise a nucleoside metabolic inhibitor.

8. The method of claim 7, wherein the one or more additional therapeutic agents is gemcitabine.

9. A method of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents, wherein the one or more additional therapeutic agents comprise a microtubule stabilizing agent.

10. The method of claim 9, wherein the microtubule stabilizing agent is selected from the group consisting of paclitaxel injection, nab-paclitaxel, cabazitaxel and docetaxel.

11. The method of claim 8, wherein the one or more additional therapeutic agents is nab-paclitaxel.

12. The method of claim 11, wherein co-administration of the anti-ErbB3 antibody and the nab-paclitaxel has an additive or superadditive effect on suppressing pancreatic tumor growth, wherein the effect on suppressing pancreatic tumor growth is measured in a mouse xenograft model using BxPC-3 or COLO-357 cells implanted as xenografts in nude mice.

13. A method of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents wherein the one or more additional therapeutic agents comprise a topoisomerase 1 inhibitor and optionally wherein the topoisomerase 1 inhibitor is formulated for intravenous administration.

14. The method of claim 13, wherein the wherein the topoisomerase 1 inhibitor is a camptothecin selected from the group consisting of 9-aminocamptothecin, 7-ethylcamptothecin, 10-hydroxycamptothecin, 9-nitrocamptothecin, 10,11-methylenedioxycamptothecin, 9-amino-10,11-methylenedioxycamptothecin, 9-chloro-10,11-methylenedioxycamptothecin, topotecan, lurtotecan, silatecan, and irinotecan.

15. The method of claim 14, wherein the camptothecin is irinotecan or topotecan and the irinotecan or topotecan is liposomally encapsulated irinotecan or liposomally encapsulated topotecan.

16. The method of claim 15, wherein the one or more additional therapeutic agents is liposomally encapsulated irinotecan or liposomally encapsulated topotecan and the liposomally encapsulated irinotecan or liposomally encapsulated topotecan is in the form of a sucrose octasulfate salt.

17. A method of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents, wherein the one or more additional therapeutic agents is chosen from the group consisting of a bispecific anti-ErbB2/anti-ErbB3 antibody, an anti-IGF-1R/anti-ErbB3 antibody, an anti EGFR/anti-ErbB3 antibody, or a mixture of anti-EGFR and anti-ErbB3 antibodies.

18. The method of claim any of claims 1 to 17, wherein the anti-ErbB3 antibody comprises CDRH1, CDRH2, and CDRH3 sequences comprising the amino acid sequences set forth in SEQ ID NO: 5 (CDRH1) SEQ ID NO: 6 (CDRH2) and SEQ ID NO: 7 (CDRH3), and further comprises CDRL1,

CDRL2, and CDRL3 sequences comprising the amino acid sequences set forth in SEQ ID NO:8 (CDRL1) SEQ ID NO: 9 (CDRL2) and SEQ ID NO: 10 (CDRL3).

19. The method of claim 18, wherein the anti-ErbB3 antibody comprises V_H and/or V_L regions comprising the amino acid sequences set forth in SEQ ID NOs: 2 and 4, respectively.

20. The method of claim any of claims 1 to 17, wherein the anti-ErbB3 antibody is selected from 8B8, 1B4C3, 2D1D12, GE-huMab-HER3, MEDI3379, AMG888 and AV-203.

21. The method of claim any one of claims 1 to 20, wherein co-administration of the anti-ErbB3 antibody and the additional chemotherapeutic agent or agents has an additive or superadditive effect on suppressing pancreatic tumor growth, as compared to administration of the anti-ErbB3 antibody alone or the one or more additional chemotherapeutic agents alone, wherein the effect on suppressing pancreatic tumor growth is measured in a mouse xenograft model using BxPC-3 or COLO-357cells.

22. The method of any one of claims 1 to 20, wherein at least one of the one or more additional therapeutic agents is administered at a dosage that comprises at least one reduced dose that provides less of the at least one additional therapeutic agent than is provided by a dosage recommended by the manufacturer of the at least one additional therapeutic agent for administration for the treatment of cancer in a patient who is not receiving concurrent anti-ErbB3 antibody therapy.

23. The method of any one of claims 4 to 6 or 17 to 20, wherein at least one of the one or more additional therapeutic agents is administered at a dosage that comprises at least one reduced dose that provides less of the one or more additional therapeutic agents than is provided by a dosage recommended by the manufacturer of the one or more additional therapeutic agents for administration for the treatment of cancer in a patient who is not receiving concurrent anti-ErbB3 antibody therapy.

24. The method of claim 22 or 23, wherein the reduced dose is about half the dose recommended by the manufacturer.

25. The method of any one of claims 1 to 24 or the composition of any one of claims 58-79, wherein the antibody is formulated for intravenous administration.

26. The method of any one of claims 1 to 24 or the composition of any one of claims 58-79, wherein the patient has recurrent or persistent pancreatic cancer following primary chemotherapy.

27. The method of any one of claims 1 to 24 or the composition of any one of claims 58-79, wherein the patient has failed prior therapy with a platinum-based therapeutic agent.

28. The method of any one of claims 1 to 24 or the composition of any one of claims 58-79, wherein the patient has failed prior treatment with, or become resistant to treatment with one or more of a) a nucleoside analog therapeutic agent, b) a platinum-based therapeutic agent, c) a therapeutic agent, that is a topoisomerase 1 inhibitor and d) a therapeutic agent that is a tyrosine kinase inhibitor.

29. The method of any one of claims 1 to 20, wherein each of the additional therapeutic agent or agents is administered following the administration of the anti-ErbB3 antibody.

30. The method of any one of 13 to 17 or 28, wherein the topoisomerase 1 inhibitor is administered before the administration of the anti-ErbB3 antibody.

31. The method of any one of claims 13 to 17 or 28, wherein the topoisomerase 1 inhibitor and the anti-ErbB3 antibody are administered simultaneously.

32. The method of any one of claims 9 to 12, wherein the microtubule stabilizing agent is formulated for intravenous administration.

33. The method of any one of claims 9 to 12, wherein the microtubule stabilizing agent is administered following the administration of the anti-ErbB3 antibody.

34. The method of any one of claims 9 to 12, wherein the microtubule stabilizing agent is administered before the administration of the anti-ErbB3 antibody.

35. The method of any one of claims 9 to 12, wherein the microtubule stabilizing agent and the anti-ErbB3 antibody are administered simultaneously.

36. The method of any one of claims 1 to 6, wherein the EGFR inhibitor is formulated for oral administration.

37. The method of claim 7 or 8, wherein the nucleoside metabolic inhibitor is formulated for intravenous administration.

38. The method of claim 7 or 8, wherein the EGFR inhibitor and the nucleoside metabolic inhibitor are administered following the administration of the anti-ErbB3 antibody.

39. The method of claim 7 or 8, wherein the EGFR inhibitor and the nucleoside metabolic inhibitor are administered before the administration of the anti-ErbB3 antibody.

40. The method of claim 7 or 8, wherein the EGFR inhibitor, the nucleoside metabolic inhibitor and the anti-ErbB3 antibody are administered simultaneously.

41. The method of any one of claims 1 to 40, wherein the one or more additional therapeutic agents comprise two or more additional therapeutic agents.

42. The method of any one of claims 1 to 6 and 9 to 41, wherein the one or more additional therapeutic agents comprise two or more additional therapeutic agents, one of which is gemcitabine.

43. The method of claim 41, wherein the two or more additional therapeutic agents is a combination of folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX).

44. The method of claim 41, wherein the two or more additional therapeutic agents is a combination of folinic acid, 5-fluorouracil, and oxaliplatin (FOLFOX).

45. The method of claim 41, wherein the one or more additional therapeutic agents is a combination of folinic acid, 5-fluorouracil, and irinotecan (FOLFIRI).

46. The method of any one of claims 1 to 45, wherein the anti-ErbB3 antibody is formulated for intravenous administration and is selected from the group comprising AMG888, AV-203, 8B8, 1B4C3 and 2D1D12.

47. The method of any one of claims 1 to 45, wherein the anti-ErbB3 antibody is formulated for intravenous administration and comprises V_H and/or V_L regions comprising the amino acid sequences set forth in SEQ ID NOs: 2 and 4, respectively.

48. The method of any one of claims 1 to 47, wherein the pancreatic cancer is an exocrine pancreatic cancer selected from the group consisting of acinar cell carcinoma, adenocarcinoma, adenosquamous carcinoma, giant cell tumor, intraductal papillary-mucinous neoplasm (IPMN), mucinous cystadenocarcinoma, pancreatoblastoma, serous cystadenocarcinoma, and solid and pseudopapillary tumors.

49. The method of claim 48, wherein the adenocarcinoma is pancreatic ductal carcinoma.

50. The method of any one of claims 1 to 47, wherein the pancreatic cancer is an endocrine pancreatic cancer selected from the group consisting of: Gastrinoma (Zollinger-Ellison Syndrome), Insulinoma, Nonfunctional Islet Cell Tumor, Somatostatinoma, and Vasoactive Intestinal Peptide-Releasing Tumor (VIPoma or Verner-Morrison Syndrome).

51. The method of any one of claims 1 to 47, wherein the pancreatic cancer comprises a KRAS gene comprising a KRAS mutation.

52. The method of claim 51, wherein the KRAS mutation is KRAS G12S.

53. The method of claim 50, wherein the one or more additional therapeutic agents comprise an mTOR inhibitor selected from the group consisting of temsirolimus, everolimus, sirolimus, and ridaforolimus.

54. The method of claim 53, wherein the mTOR inhibitor is everolimus.

55. The method of any one of claims 1 to 47, wherein the pancreatic cancer comprises a BRAF gene comprising a BRAF mutation and optionally wherein one of the one or more additional therapeutic agents is a BRAF kinase inhibitor, optionally vemurafenib.

56. The method of claim 55, wherein the BRAF mutation is BRAF V600E.

57. The method of any one of claims 1 to 56, wherein the treatment produces at least one therapeutic effect selected from the group consisting of: reduction of the rate of tumor growth, reduction in size of tumor, reduction of tumor mitotic index, reduction in number of metastatic lesions over time, complete response, partial response, stable disease, increase in overall response rate, or a pathologic complete response.

58. A composition for the treatment of pancreatic cancer, or for the manufacture of a medicament for the treatment of pancreatic cancer, in a patient, said treatment comprising co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents, wherein the one of the one or more additional therapeutic agents comprise an EGFR inhibitor.

59. The composition of claim 58, wherein the one or more additional therapeutic agents is an EGFR inhibitor that is selected from, gefitinib, erlotinib, afatinib and lapatinib.

60. The composition of claim 58, wherein the one or more additional therapeutic agents is an EGFR inhibitor that is selected from, MM-151, Sym004, cetuximab, panitumumab, zalutumumab, nimotuzumab, and matuzumab.

61. The composition of claim 58, wherein the one or more additional therapeutic agents further comprise a nucleoside metabolic inhibitor.

62. The composition of claim 61, wherein the nucleoside metabolic inhibitor is gemcitabine.

63. The composition of claim 62, wherein the EGFR inhibitor is erlotinib.

64. A composition for the treatment of pancreatic cancer, or for the manufacture of a medicament for the treatment of pancreatic cancer, in a patient, said treatment comprising co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents wherein the one or more additional therapeutic agents comprise a nucleoside metabolic inhibitor.

65. The composition of claim 64, wherein the one or more additional therapeutic agents is gemcitabine.

66. A composition for the treatment of pancreatic cancer, or for the manufacture of a medicament for the treatment of pancreatic cancer, in a patient, said treatment comprising co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents wherein the one or more additional therapeutic agents comprise a microtubule stabilizing agent.

67. The composition of claim 66, wherein the microtubule stabilizing agent is selected from the group consisting of paclitaxel injection, nab-paclitaxel and docetaxel.

68. The composition of claim 65, wherein the one or more additional therapeutic agents is nab-paclitaxel.

69. The composition of claim 68, wherein co-administration of the anti-ErbB3 antibody and the nab-paclitaxel has an additive or superadditive effect on suppressing pancreatic tumor growth, as compared to administration of the anti-ErbB3 antibody alone or the nab-paclitaxel alone, wherein the effect on suppressing pancreatic tumor growth is measured in a mouse xenograft model using BxPC-3 or COLO-357 cells.

70. A composition for the treatment of pancreatic cancer, or for the manufacture of a medicament for the treatment of pancreatic cancer, in a patient, said treatment comprising co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents wherein the one or more additional therapeutic agents comprise a topoisomerase 1 inhibitor.

71. The composition of claim 70, wherein the wherein the topoisomerase 1 inhibitor is a camptothecin selected from the group consisting of 9-aminocamptothecin, 7-ethylcamptothecin, 10-hydroxycamptothecin, 9-nitrocamptothecin, 10,11-methylenedioxycamptothecin, 9-amino-10,11-methylenedioxycamptothecin, 9-chloro-10,11-methylenedioxycamptothecin, topotecan, lurtotecan, silatecan, and irinotecan.

72. The composition of claim 71, wherein the camptothecin is irinotecan or topotecan and the irinotecan or topotecan is liposomally encapsulated irinotecan or liposomally encapsulated topotecan.

73. The composition of claim 72, wherein the one or more additional therapeutic agents is liposomally encapsulated irinotecan or liposomally encapsulated topotecan and the liposomally encapsulated irinotecan or liposomally encapsulated topotecan is in the form of a sucrose octasulfate salt.

74. The composition of any of claims 58 to 73, wherein the ErbB3 inhibitor is an anti-ErbB3 antibody.

75. The composition of claim 74, wherein anti-ErbB3 antibody comprises CDRH1, CDRH2, and CDRH3 sequences comprising the amino acid sequences set forth in SEQ ID NO: 5 (CDRH1) SEQ ID NO: 6 (CDRH2) and SEQ ID NO: 7 (CDRH3), and further comprises CDRL1, CDRL2, and CDRL3 sequences comprising the amino acid sequences set forth in SEQ ID NO: 8 (CDRL1) SEQ ID NO: 9 (CDRL2) and SEQ ID NO: 10 (CDRL3).

76. The composition of claim 75, wherein the anti-ErbB3 antibody comprises V_H and/or V_L regions comprising the amino acid sequences set forth in SEQ ID NOs: 2 and 4, respectively.

77. The composition of claim 74, wherein the anti-ErbB3 antibody is selected from 8B8, 1B4C3, 2D1D12, AMG888 and AV-203.

78. The composition of any one of claims 58 to 68 and 70 to 77, wherein co-administration of the anti-ErbB3 antibody and the additional chemotherapeutic agent or agents has an additive or superadditive effect on suppressing pancreatic tumor growth, as compared to administration of the anti-ErbB3 antibody alone or the one or more additional chemotherapeutic agents alone, wherein the effect on suppressing pancreatic tumor growth is measured in a mouse xenograft model using BxPC-3 or COLO-357 cells.

79. The composition of any one of claims 58 to 77, wherein at least one of the one or more additional therapeutic agents is administered at a dosage that is a reduced dose that provides less of the at least one additional therapeutic agent than is provided by a dosage recommended by the manufacturer of the at least one additional therapeutic agent for administration for the treatment of pancreatic cancer in a patient who is not receiving concurrent anti-ErbB3 antibody therapy.

80. The composition of any one of claims 61 to 63 or 74 to 77, wherein at least one of the one or more additional therapeutic agents is administered at a dosage that is a reduced dose that provides less of the one or more additional therapeutic agents than is provided by a dosage recommended by the manufacturer of the one or more additional therapeutic agents for administration for the treatment of pancreatic cancer in a patient who is not receiving concurrent anti-ErbB3 antibody therapy.

81. The composition of claim 79 or 80, wherein the reduced dose is a dose that is about half the dosage recommended by the manufacturer.

82. The composition of any one of claims 58 to 81, wherein the patient has recurrent or persistent pancreatic cancer following primary chemotherapy.

83. The composition of any one of claims 58 to 77, wherein each of the additional therapeutic agent or agents is administered following the administration of the anti-ErbB3 antibody.

84. The composition of any one of claims 70 to 74, wherein the topoisomerase 1 inhibitor is administered before the administration of the anti-ErbB3 antibody.

85. The composition of any one of claims 70 to 74, wherein the topoisomerase 1 inhibitor and the anti-ErbB3 antibody are administered simultaneously.

86. The composition of any one of claims 66 to 69, wherein the microtubule stabilizing agent is formulated for intravenous administration.

87. The composition of any one of claims 66 to 69, wherein the microtubule stabilizing agent is administered following the administration of the anti-ErbB3 antibody.

88. The composition of any one of claims 66 to 69, wherein the microtubule stabilizing agent is administered before the administration of the anti-ErbB3 antibody.

89. The composition of any one of claims 66 to 69, wherein the microtubule stabilizing agent and the anti-ErbB3 antibody are administered simultaneously.

90. The composition of any one of claims 58 to 63, wherein the EGFR inhibitor is formulated for oral administration.

91. The composition of claim 64 or 65, wherein the nucleoside metabolic inhibitor is formulated for intravenous administration.

92. The composition of claim 64 or 65, wherein the EGFR inhibitor and the nucleoside metabolic inhibitor are administered following the administration of the anti-ErbB3 antibody.

93. The composition of claim 64 or 65, wherein the EGFR inhibitor and the nucleoside metabolic inhibitor are administered before the administration of the anti-ErbB3 antibody.

94. The composition of claim 64 or 65, wherein the EGFR inhibitor, the nucleoside metabolic inhibitor and the anti-ErbB3 antibody are administered simultaneously.

95. The composition of any one of claims 58 to 97, wherein the one or more additional therapeutic agents comprise two or more additional therapeutic agents.

96. The composition of any one of claims 58 to 63 and 66 to 96, wherein the one or more additional therapeutic agents comprise two or more additional therapeutic agents, one of which is gemcitabine.

97. The composition of claim 95, wherein the two or more additional therapeutic agents is a combination of folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX).

98. The composition of claim 95, wherein the two or more additional therapeutic agents is a combination of folinic acid, 5-fluorouracil, and oxaliplatin (FOLFOX).

99. The composition of claim 95, wherein the one or more additional therapeutic agents is a combination of folinic acid, 5-fluorouracil, and irinotecan (FOLFIRI).

100. The composition of any one of claims 58 to 99, wherein the anti-ErbB3 antibody is selected from the group comprising AMG888, AV-203, 8B8, 1B4C3 and 2D1D12.

101. The composition of any one of claims 58 to 99, wherein the anti-ErbB3 antibody comprises V_H and/or V_L regions comprising the amino acid sequences set forth in SEQ ID NOs: 2 and 4, respectively.

102. The composition of any one of claims 58 to 101, wherein the pancreatic cancer is an exocrine pancreatic cancer selected from the group consisting of acinar cell carcinoma, adenocarcinoma, adenosquamous carcinoma, giant cell tumor, intraductal papillary-mucinous neoplasm (IPMN), mucinous

cystadenocarcinoma, pancreatoblastoma, serous cystadenocarcinoma, and solid and pseudopapillary tumors.

103. The composition of claim 102, wherein the adenocarcinoma is pancreatic ductal carcinoma.

104. The composition of any one of claims 58 to 101, wherein the pancreatic cancer is an endocrine pancreatic cancer selected from the group consisting of: Gastrinoma (Zollinger-Ellison Syndrome), Insulinoma, Nonfunctional Islet Cell Tumor, Somatostatinoma, and Vasoactive Intestinal Peptide-Releasing Tumor (VIPoma or Verner-Morrison Syndrome).

105. The composition of any one of claims 58 to 101, wherein the pancreatic cancer comprises a KRAS gene comprising a KRAS mutation.

106. The composition of claim 105, wherein the KRAS mutation is KRAS G12S.

107. The composition of claim 104, wherein the one or more additional therapeutic agents comprise an mTOR inhibitor selected from the group consisting of everolimus, sirolimus, and ridaforolimus.

108. The composition of claim 107, wherein the mTOR inhibitor is everolimus.

109. The composition of any one of claims 58 to 101, wherein the pancreatic cancer comprises a BRAF gene comprising a BRAF mutation and optionally wherein one of the one or more additional therapeutic agents is a BRAF kinase inhibitor, optionally vemurafenib.

110. The composition of claim 109, wherein the BRAF mutation is BRAF V600E.

111. The composition of any one of claims 58 to 110, wherein the treatment produces at least one therapeutic effect selected from the group consisting of: reduction of the rate of tumor growth, reduction in size of tumor, reduction of tumor mitotic index, reduction in number of metastatic lesions over time, complete response, partial response, stable disease, increase in overall response rate, or a pathologic complete response.

112. A method of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents,

wherein i) the antibody is an antibody having the heavy chain sequence set forth in SEQ ID NO:2 and the light chain sequence set forth in SEQ ID NO:4, and ii) the one of the one or more additional therapeutic agents comprises erlotinib.

113. A method of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents, wherein i) the antibody is an antibody having the heavy chain sequence set forth in SEQ ID NO:2 and the light chain sequence set forth in SEQ ID NO:4, and ii) the one of the one or more additional therapeutic agents comprises erlotinib and gemcitabine.

114. A method of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents, wherein i) the antibody is an antibody having the heavy chain sequence set forth in SEQ ID NO:2 and the light chain sequence set forth in SEQ ID NO:4, and ii) the one of the one or more additional therapeutic agents comprises everolimus.

115. A method of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents, wherein i) the antibody is an antibody having the heavy chain sequence set forth in SEQ ID NO:2 and the light chain sequence set forth in SEQ ID NO:4, and ii) the one of the one or more additional therapeutic agents comprises irinotecan.

116. A method of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents, wherein i) the antibody is an antibody having the heavy chain sequence set forth in SEQ ID NO:2 and the light chain sequence set forth in SEQ ID NO:4, and ii) the one of the one or more additional therapeutic agents comprises everolimus and exemestane.

117. A method of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents, wherein i) the antibody is an antibody having the heavy chain sequence set forth in SEQ ID NO:2 and the light chain sequence set forth in SEQ ID NO:4, and ii) the one of the one or more additional therapeutic agents comprises nab-paclitaxel.

118. A method of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents, wherein i) the antibody is an antibody having the heavy chain sequence set forth in SEQ ID NO:2 and the light chain sequence set forth in SEQ ID NO:4, and ii) the one of the one or more additional therapeutic agents comprises cetuximab.

119. A method of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents, wherein i) the antibody is an antibody having the heavy chain sequence set forth in SEQ ID NO:2 and the light chain sequence set forth in SEQ ID NO:4, and ii) the one of the one or more additional therapeutic agents comprises MM-151.

120. A method of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents, wherein i) the antibody is an antibody having the heavy chain sequence set forth in SEQ ID NO:2 and the light chain sequence set forth in SEQ ID NO:4, and ii) the one of the one or more additional therapeutic agents comprises gemcitabine.

121. A method of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents, wherein i) the antibody is an antibody having the heavy chain sequence set forth in SEQ ID NO:2 and the light chain sequence set forth in SEQ ID NO:4, and ii) the one of the one or more additional therapeutic agents comprises eribulin.

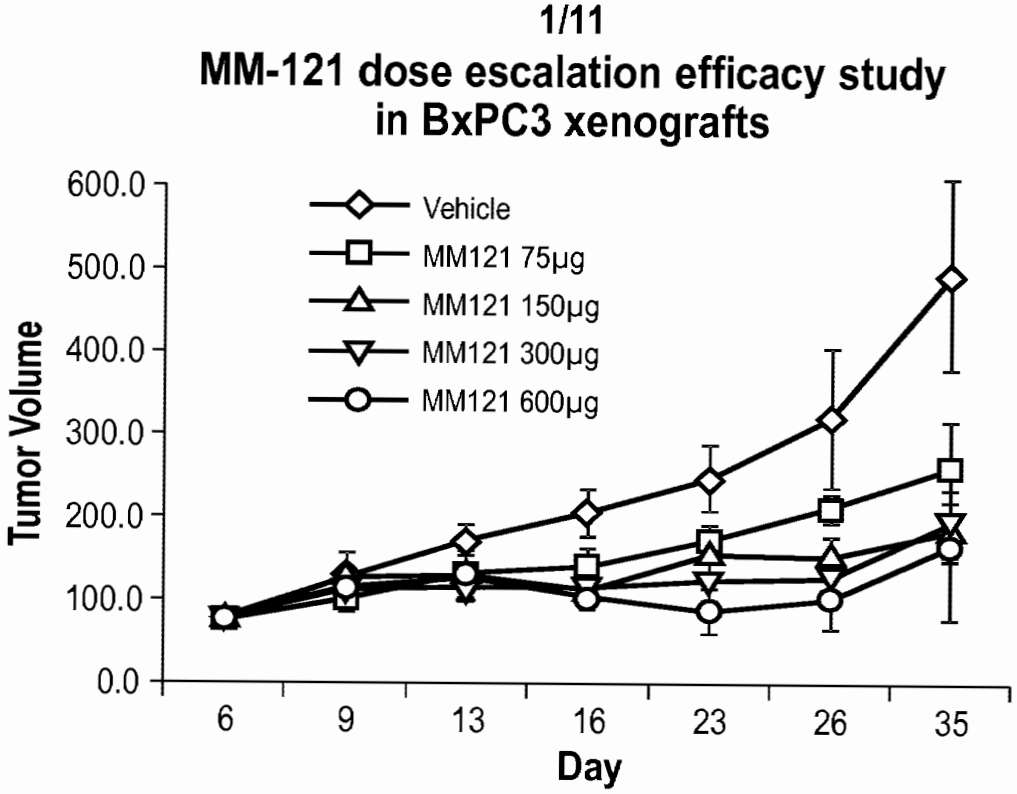

MM-121 dose escalation efficacy study
in BxPC3 xenografts
Vehicle
MM121 75µg
MM121 150µg
MM121 300µg
MM121 600µg
Tumor Volume
600.0
500.0
400.0
300.0
200.0
100.0
0.0
6
9
13
16
23
26
35
Day

Fig. 1A

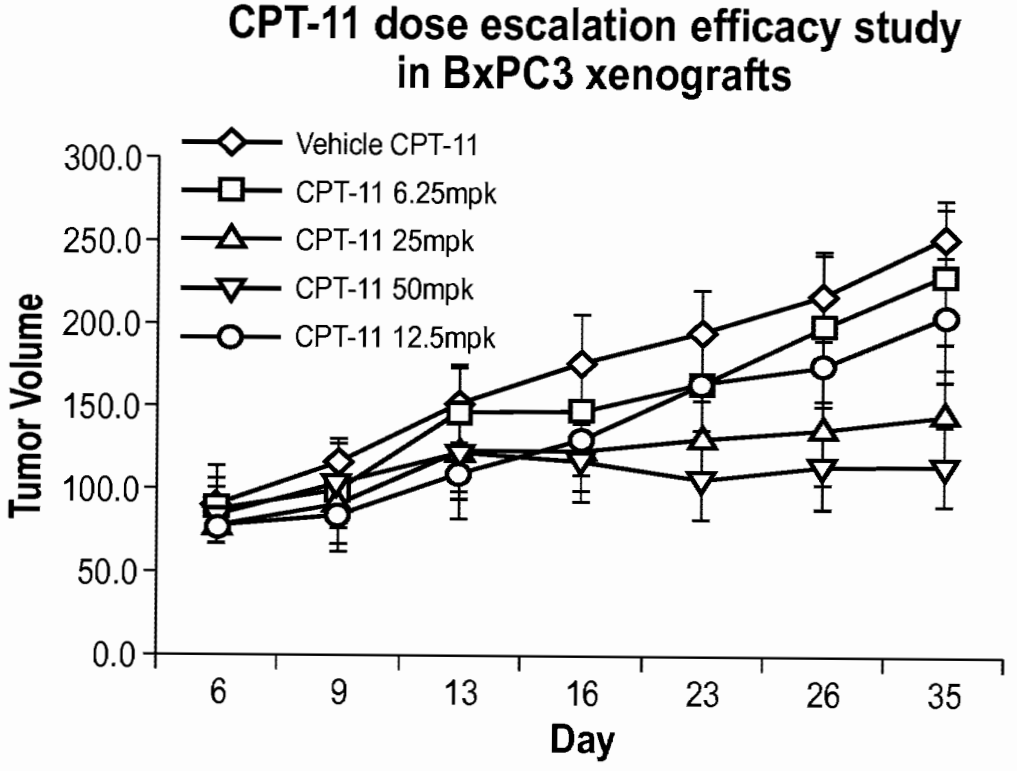

CPT-11 dose escalation efficacy study
in BxPC3 xenografts
Vehicle CPT-11
CPT-11 6.25mpk
CPT-11 25mpk
CPT-11 50mpk
CPT-11 12.5mpk
Tumor Volume
300.0
250.0
200.0
150.0
100.0
50.0
0.0
6
9
13
16
23
26
35
Day

Fig. 1B

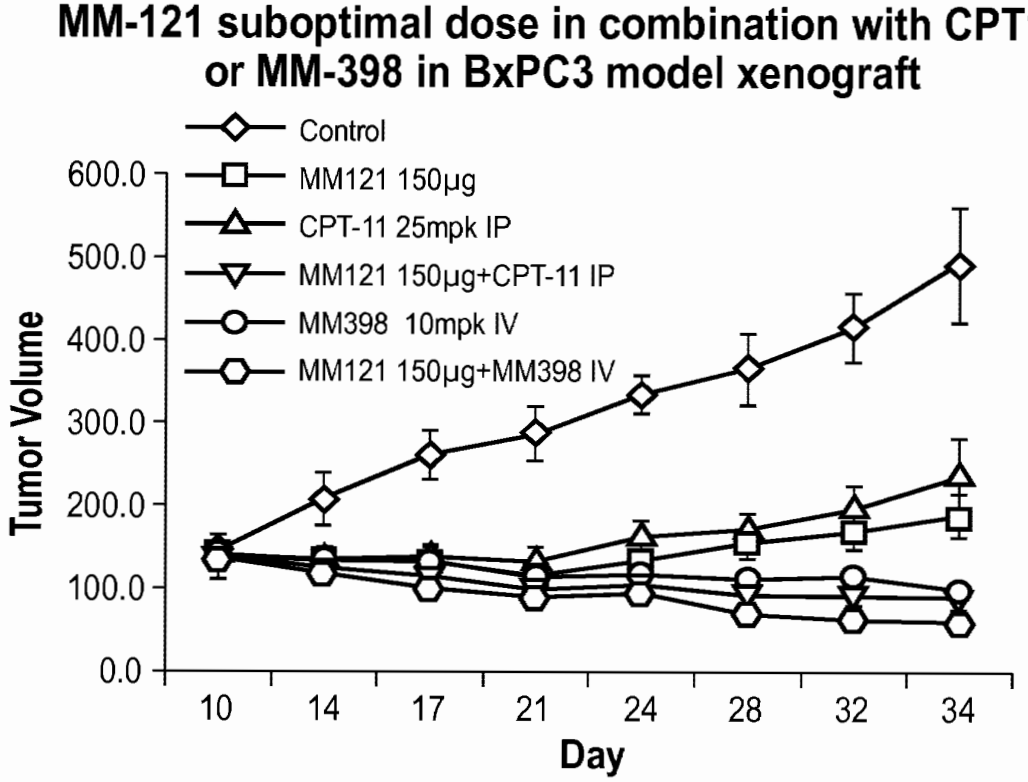

MM-121 suboptimal dose in combination with CPT11 or MM-398 in BxPC3 model xenograft
Control
MM121 150µg
CPT-11 25mpk IP
MM121 150µg+CPT-11 IP
MM398 10mpk IV
MM121 150µg+MM398 IV
Tumor Volume
600.0
500.0
400.0
300.0
200.0
100.0
0.0
10
14
17
21
24
28
32
34
Day

Fig. 2A

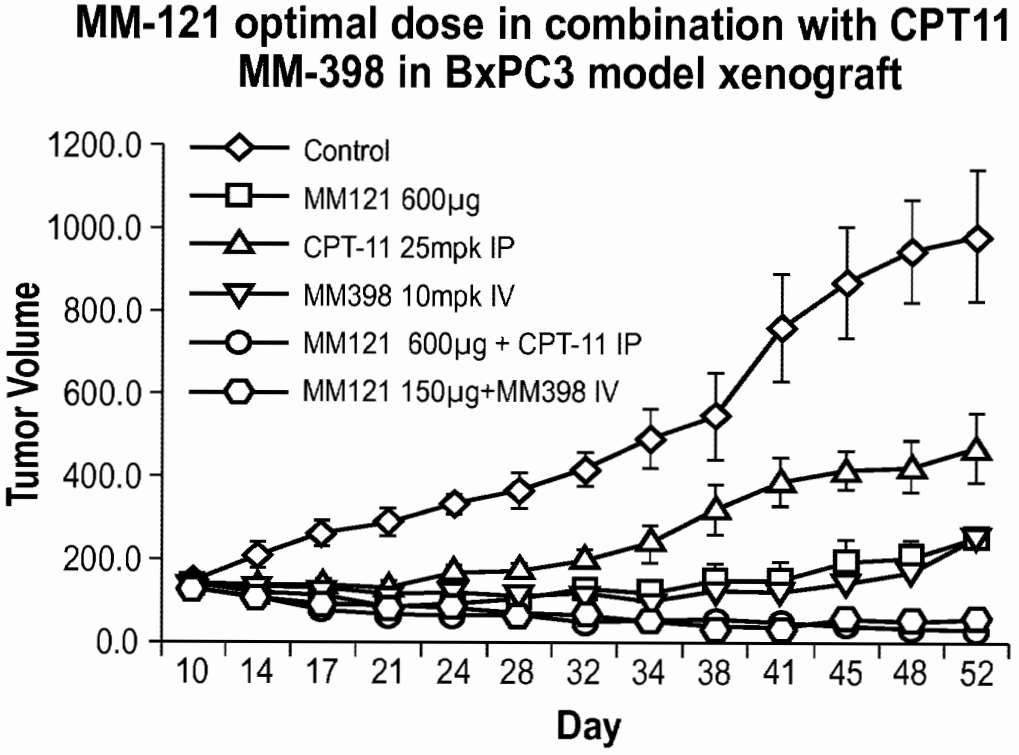

MM-121 optimal dose in combination with CPT11 or MM-398 in BxPC3 model xenograft
Control
MM121 600µg
CPT-11 25mpk IP
MM398 10mpk IV
MM121 600µg + CPT-11 IP
MM121 150µg+MM398 IV
Tumor Volume
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
10
14
17
21
24
28
32
34
38
41
45
48
52
Day

Fig. 2B

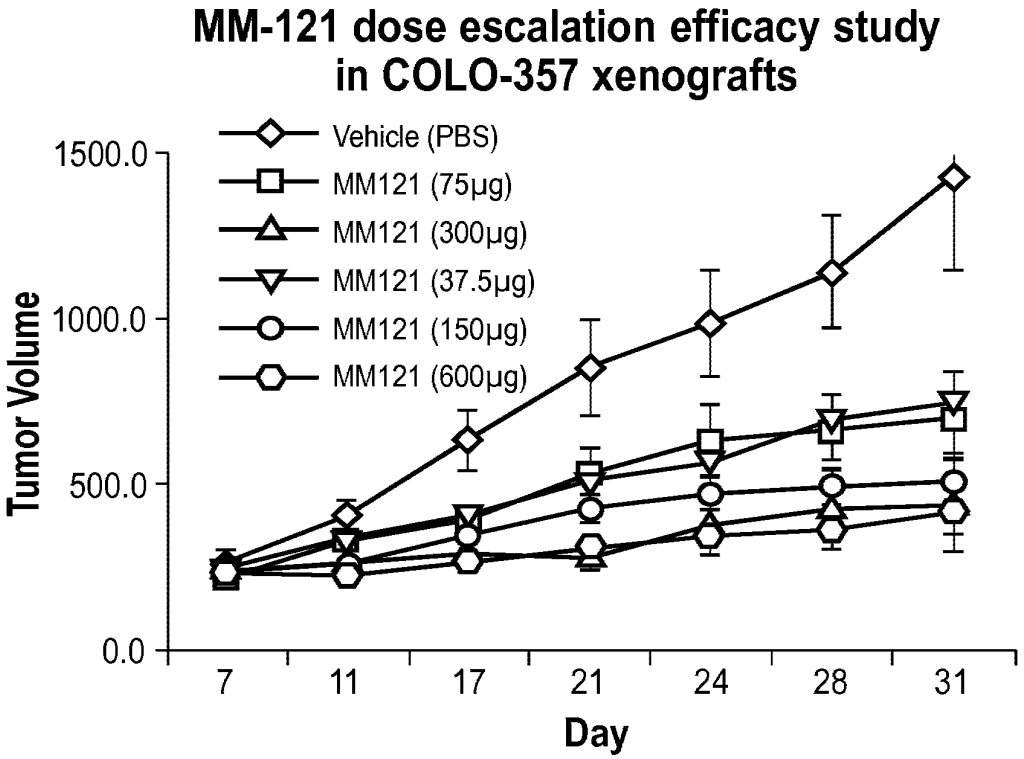

MM-121 dose escalation efficacy study
in COLO-357 xenografts
Vehicle (PBS)
MM121 (75µg)
MM121 (300µg)
MM121 (37.5µg)
MM121 (150µg)
MM121 (600µg)
Tumor Volume
1500.0
1000.0
500.0
0.0
7
11
17
21
24
28
31
Day

Fig. 3

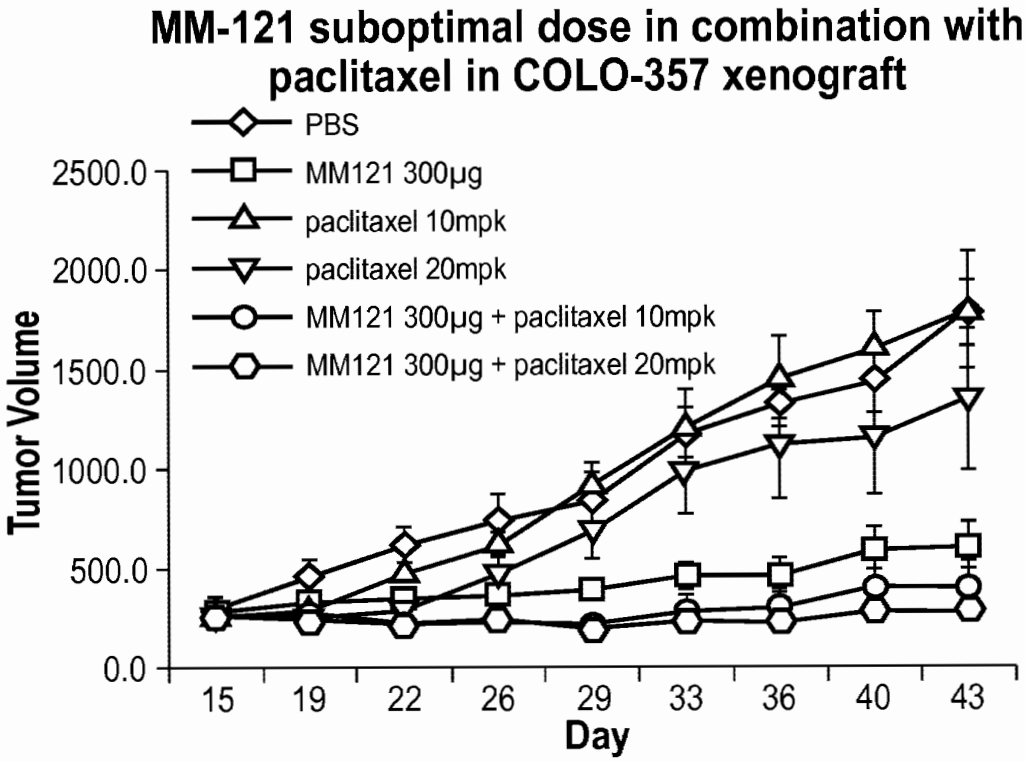

MM-121 suboptimal dose in combination with paclitaxel in COLO-357 xenograft
PBS
MM121 300µg
paclitaxel 10mpk
paclitaxel 20mpk
MM121 300µg + paclitaxel 10mpk
MM121 300µg + paclitaxel 20mpk
Tumor Volume
2500.0
2000.0
1500.0
1000.0
500.0
0.0
15
19
22
26
29
33
36
40
43
Day

Fig. 4A

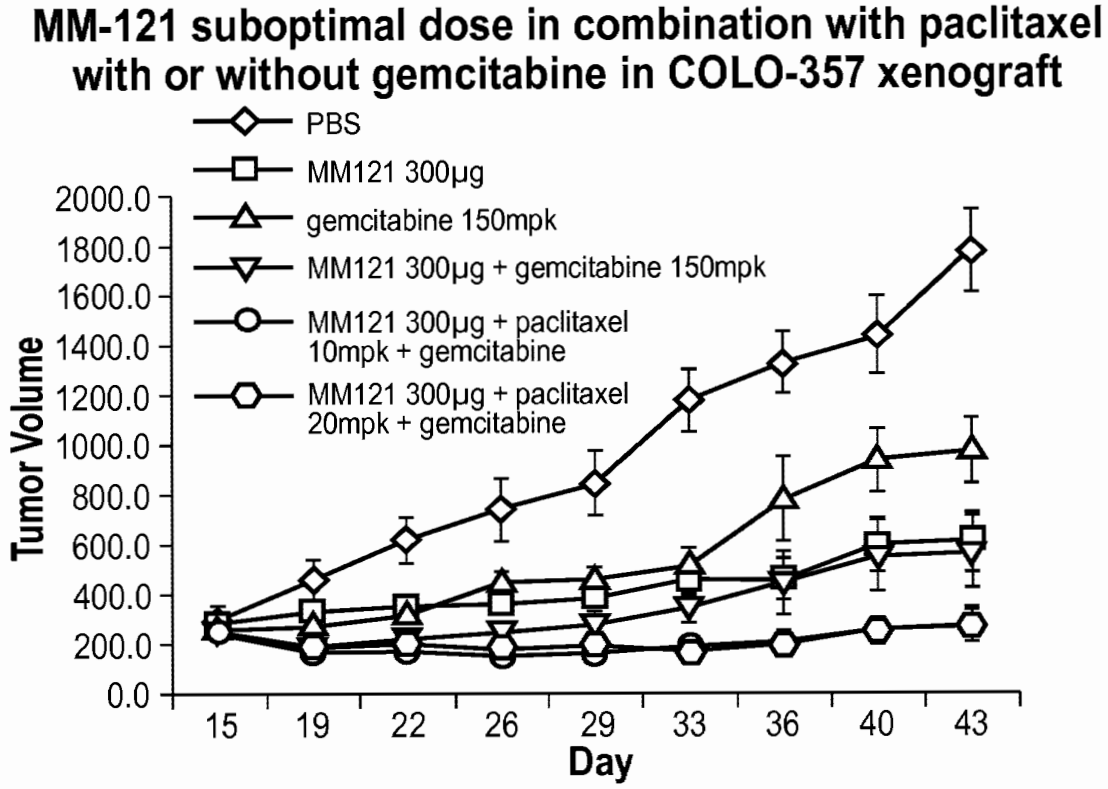
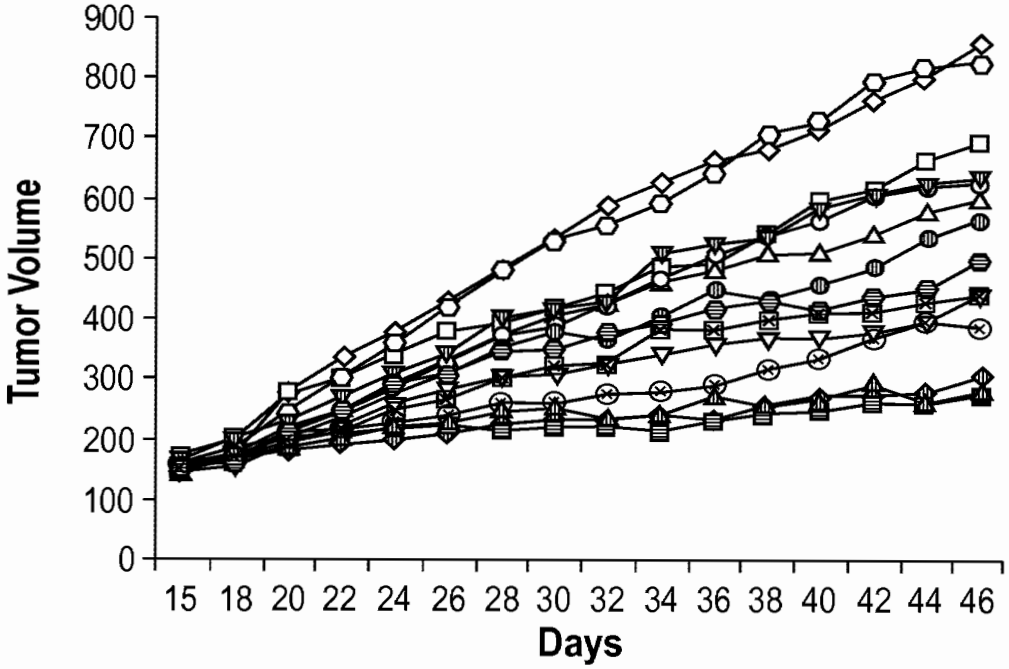

MM-121 suboptimal dose in combination with paclitaxel with or without gemcitabine in COLO-357 xenograft
PBS
MM121 300µg
gemcitabine 150mpk
MM121 300µg + gemcitabine 150mpk
MM121 300µg + paclitaxel 10mpk + gemcitabine
MM121 300µg + paclitaxel 20mpk + gemcitabine
Tumor Volume
2000.0
1800.0
1600.0
1400.0
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
15
19
22
26
29
33
36
40
43
Day

Fig. 4B

MM-121 suboptimal dose in combination with erlotinib and gemcitabine in COLO-357 xenograft (first graph with all groups included followed by graphs with each distinct dose combination)



- Group 1 - PBS Vehicle Control
- Group 2 - MM121 (300µg)
- Group 3 - erlotinib (50mpk)
- Group 4 - erlotinib (100mpk)
- Group 5 - gemcitabine (150mpk)
- Group 6 - gemcitabine (300mpk)
- Group 7 - MM121 (300µg), erlotinib (50mpk), gemcitabine (150mpk)
- Group 8 - MM121 (300µg), erlotinib (50mpk), gemcitabine (300mpk)
- Group 9 – MM121 (300µg), erlotinib (100mpk), gemcitabine (150mpk)
- Group 10 – MM121 (300µg), erlotinib (100mpk), gemcitabine (300mpk)
- Group 11 – erlotinib (50mpk), gemcitabine (150mpk)
- Group 12 – erlotinib (100mpk), gemcitabine (300mpk)
- Group 13 – erlotinib (50mpk), gemcitabine (300mpk)
- Group 14 – erlotinib (100mpk), gemcitabine (150mpk)

Fig. 5A

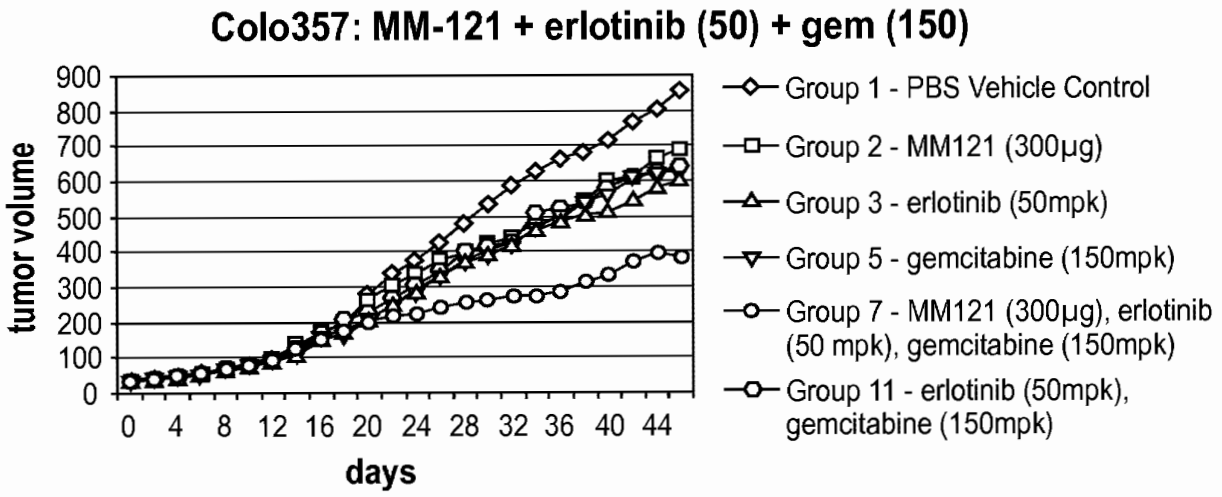

Colo357: MM-121 + erlotinib (50) + gem (150)
tumor volume
900
800
700
600
500
400
300
200
100
0
0 4 8 12 16 20 24 28 32 36 40 44
days
Group 1 - PBS Vehicle Control
Group 2 - MM121 (300µg)
Group 3 - erlotinib (50mpk)
Group 5 - gemcitabine (150mpk)
Group 7 - MM121 (300µg), erlotinib (50 mpk), gemcitabine (150mpk)
Group 11 - erlotinib (50mpk), gemcitabine (150mpk)

Fig. 5B

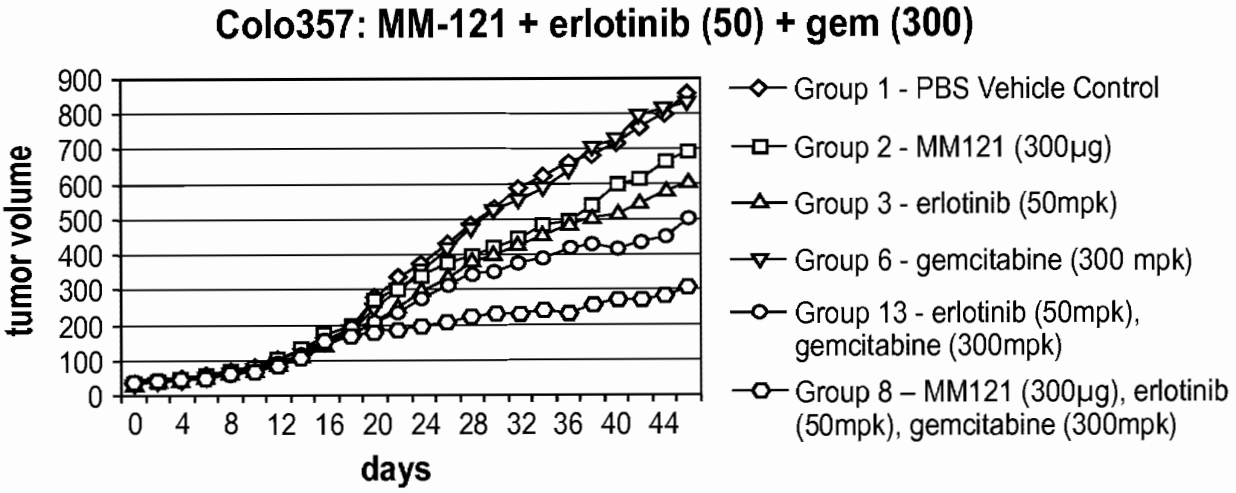

Colo357: MM-121 + erlotinib (50) + gem (300)
tumor volume
900
800
700
600
500
400
300
200
100
0
0 4 8 12 16 20 24 28 32 36 40 44
days
Group 1 - PBS Vehicle Control
Group 2 - MM121 (300µg)
Group 3 - erlotinib (50mpk)
Group 6 - gemcitabine (300 mpk)
Group 13 - erlotinib (50mpk), gemcitabine (300mpk)
Group 8 – MM121 (300µg), erlotinib (50mpk), gemcitabine (300mpk)

Fig. 5C

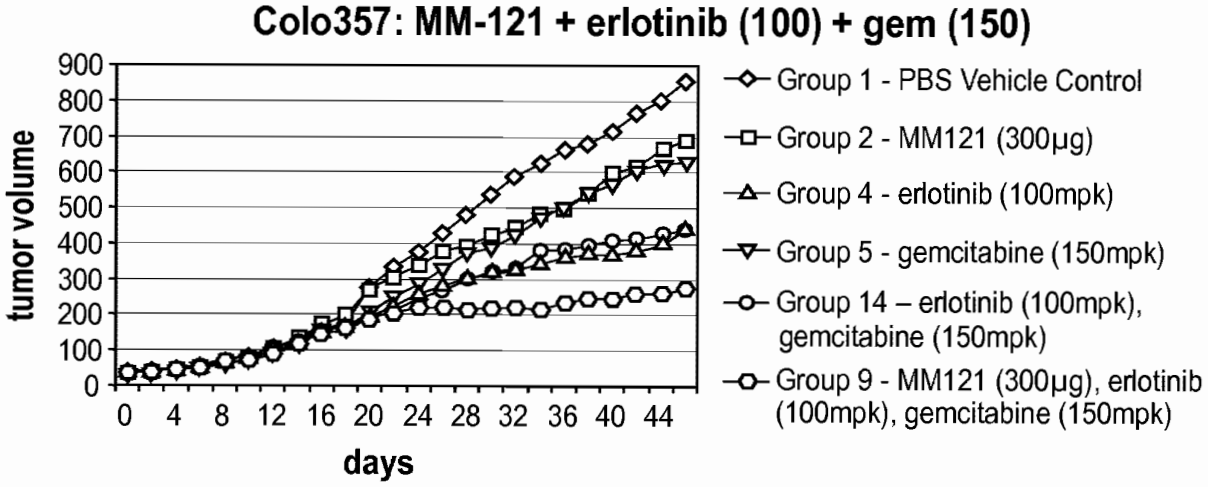

Colo357: MM-121 + erlotinib (100) + gem (150)
tumor volume
900
800
700
600
500
400
300
200
100
0
0 4 8 12 16 20 24 28 32 36 40 44
days
Group 1 - PBS Vehicle Control
Group 2 - MM121 (300µg)
Group 4 - erlotinib (100mpk)
Group 5 - gemcitabine (150mpk)
Group 14 – erlotinib (100mpk), gemcitabine (150mpk)
Group 9 - MM121 (300µg), erlotinib (100mpk), gemcitabine (150mpk)

Fig. 5D

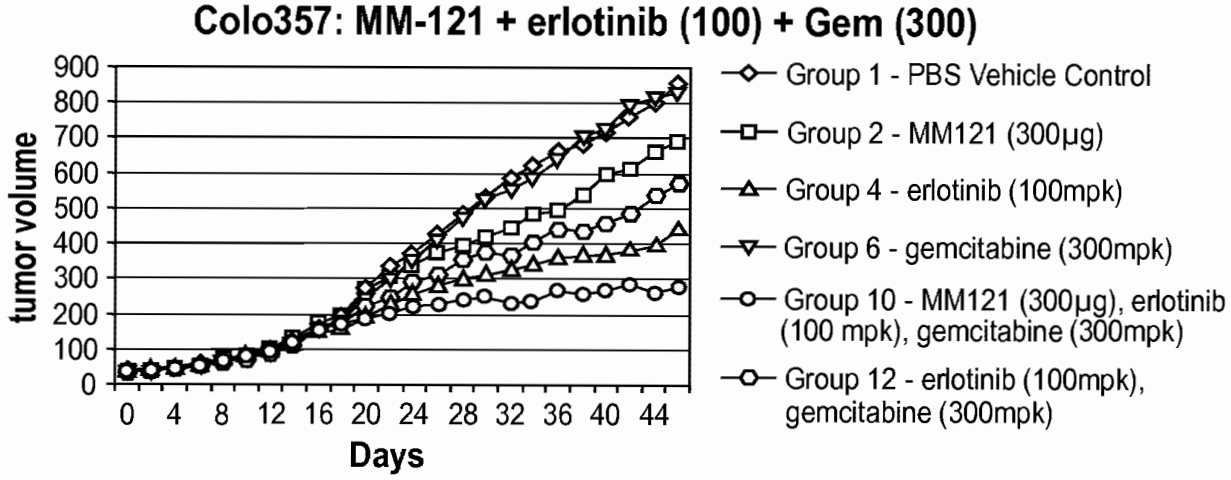

Colo357: MM-121 + erlotinib (100) + Gem (300)
tumor volume
900
800
700
600
500
400
300
200
100
0
0 4 8 12 16 20 24 28 32 36 40 44
Days
Group 1 - PBS Vehicle Control
Group 2 - MM121 (300µg)
Group 4 - erlotinib (100mpk)
Group 6 - gemcitabine (300mpk)
Group 10 - MM121 (300µg), erlotinib (100 mpk), gemcitabine (300mpk)
Group 12 - erlotinib (100mpk), gemcitabine (300mpk)

Fig. 5E

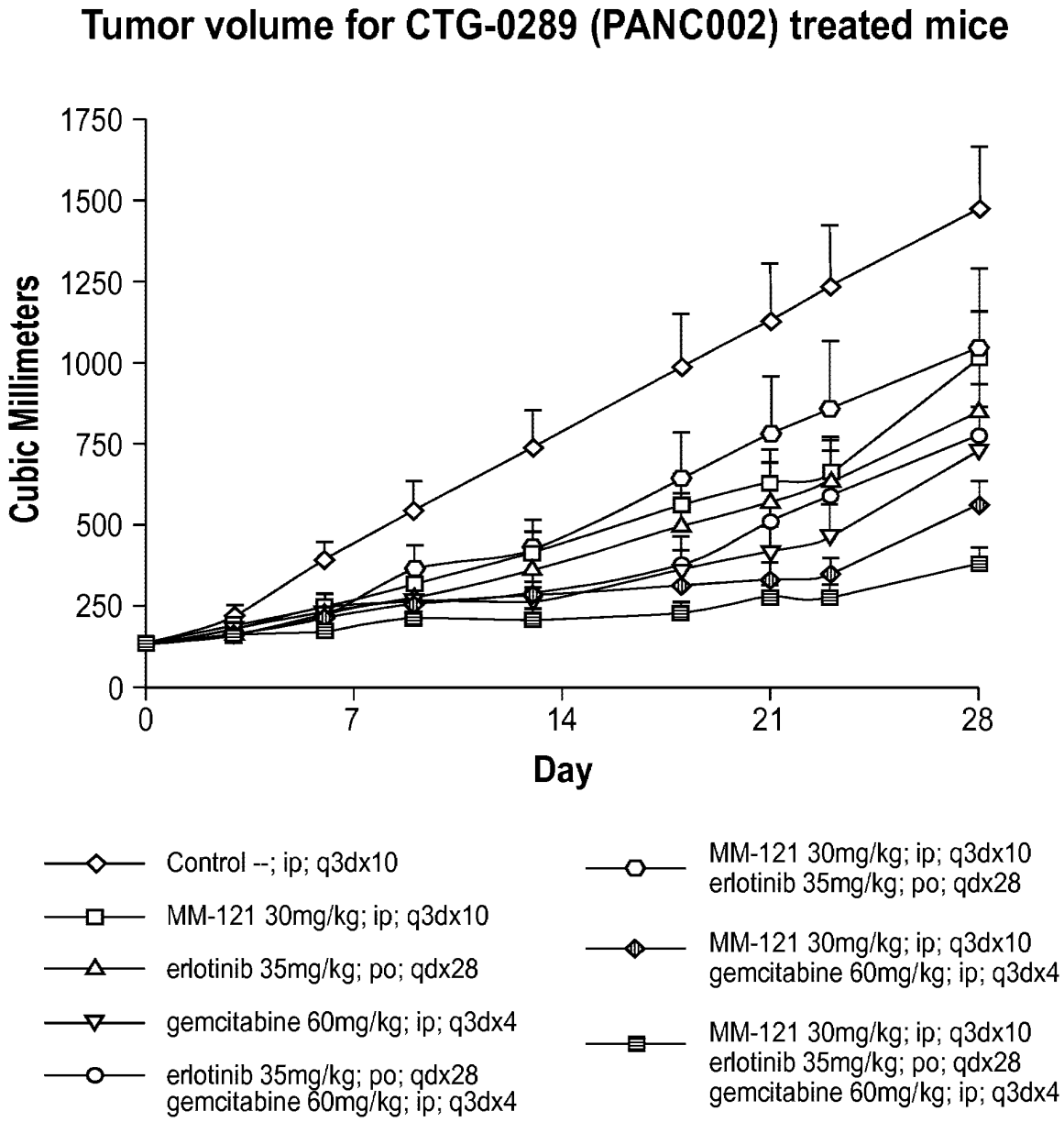

Tumor volume for CTG-0289 (PANC002) treated mice
Cubic Millimeters
1750
1500
1250
1000
750
500
250
0
0
7
14
21
28
Day
Control --; ip; q3dx10
MM-121 30mg/kg; ip; q3dx10
erlotinib 35mg/kg; po; qdx28
gemcitabine 60mg/kg; ip; q3dx4
erlotinib 35mg/kg; po; qdx28
gemcitabine 60mg/kg; ip; q3dx4
MM-121 30mg/kg; ip; q3dx10
erlotinib 35mg/kg; po; qdx28
MM-121 30mg/kg; ip; q3dx10
gemcitabine 60mg/kg; ip; q3dx4
MM-121 30mg/kg; ip; q3dx10
erlotinib 35mg/kg; po; qdx28
gemcitabine 60mg/kg; ip; q3dx4

Fig. 6

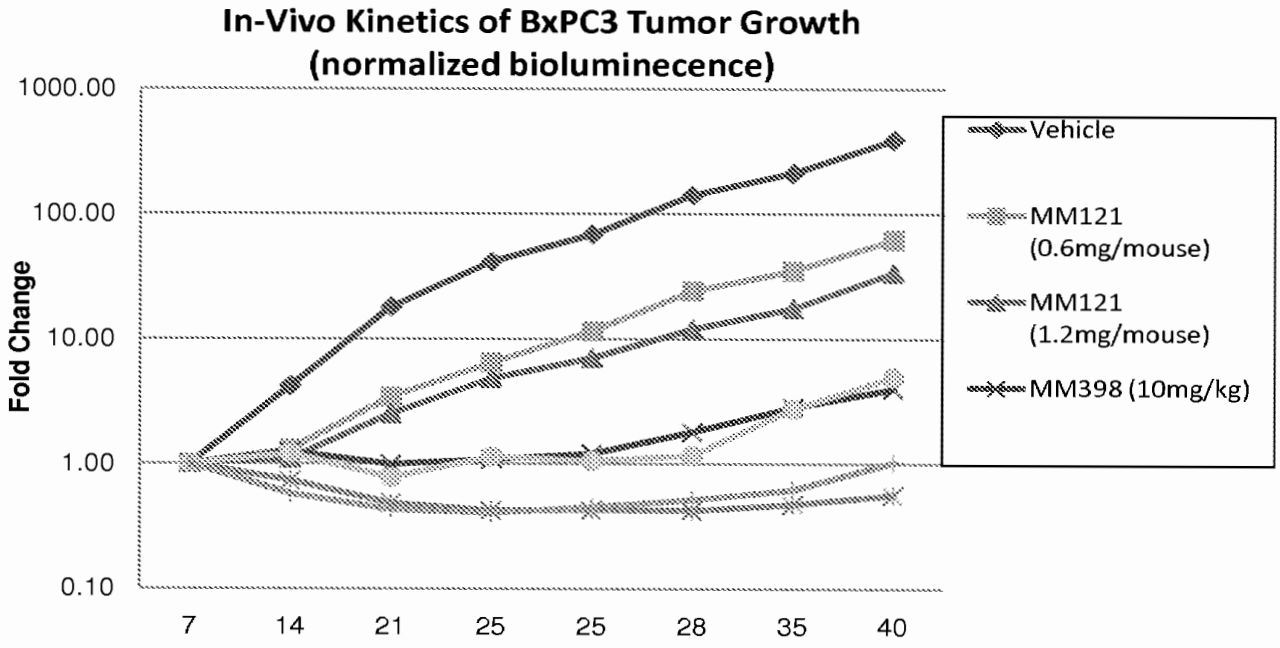

In-Vivo Kinetics of BxPC3 Tumor Growth
(normalized bioluminecence)
Fold Change
1000.00
100.00
10.00
1.00
0.10
7
14
21
25
25
28
35
40
Vehicle
MM121
(0.6mg/mouse)
MM121
(1.2mg/mouse)
MM398 (10mg/kg)

Fig. 7

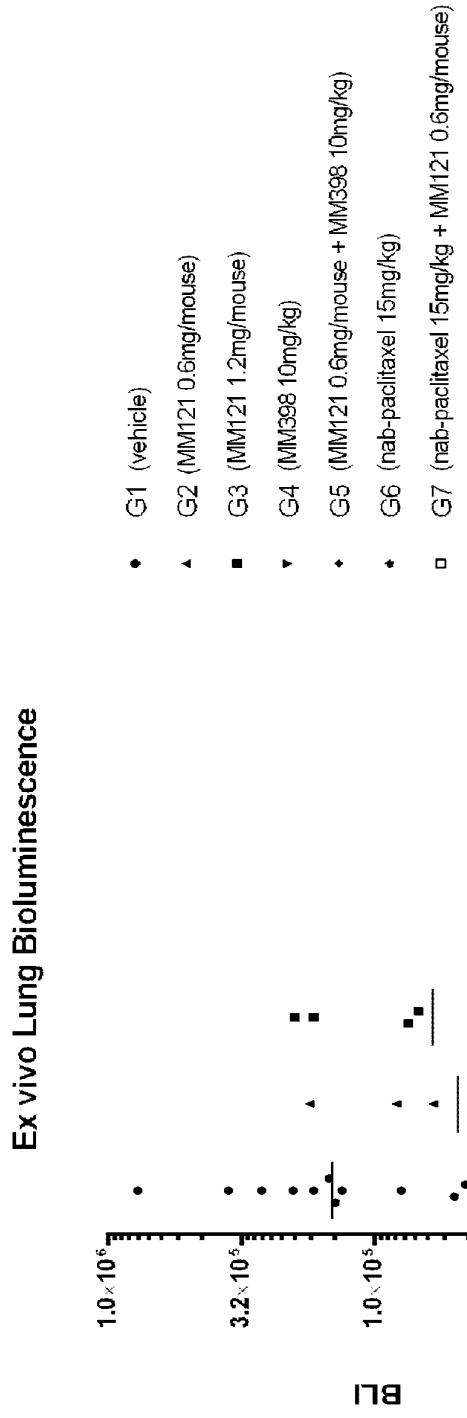

Ex vivo Lung Bioluminescence
BLI
1.0×10^6
3.2×10^5
1.0×10^5
3.2×10^4
1.0×10^4
G1
G2
G3
G4
G5
G6
G7
Treatment Groups
G1 (vehicle)
G2 (MM121 0.6mg/mouse)
G3 (MM121 1.2mg/mouse)
G4 (MM398 10mg/kg)
G5 (MM121 0.6mg/mouse + MM398 10mg/kg)
G6 (nab-paclitaxel 15mg/kg)
G7 (nab-paclitaxel 15mg/kg + MM121 0.6mg/mouse)

Fig. 8A

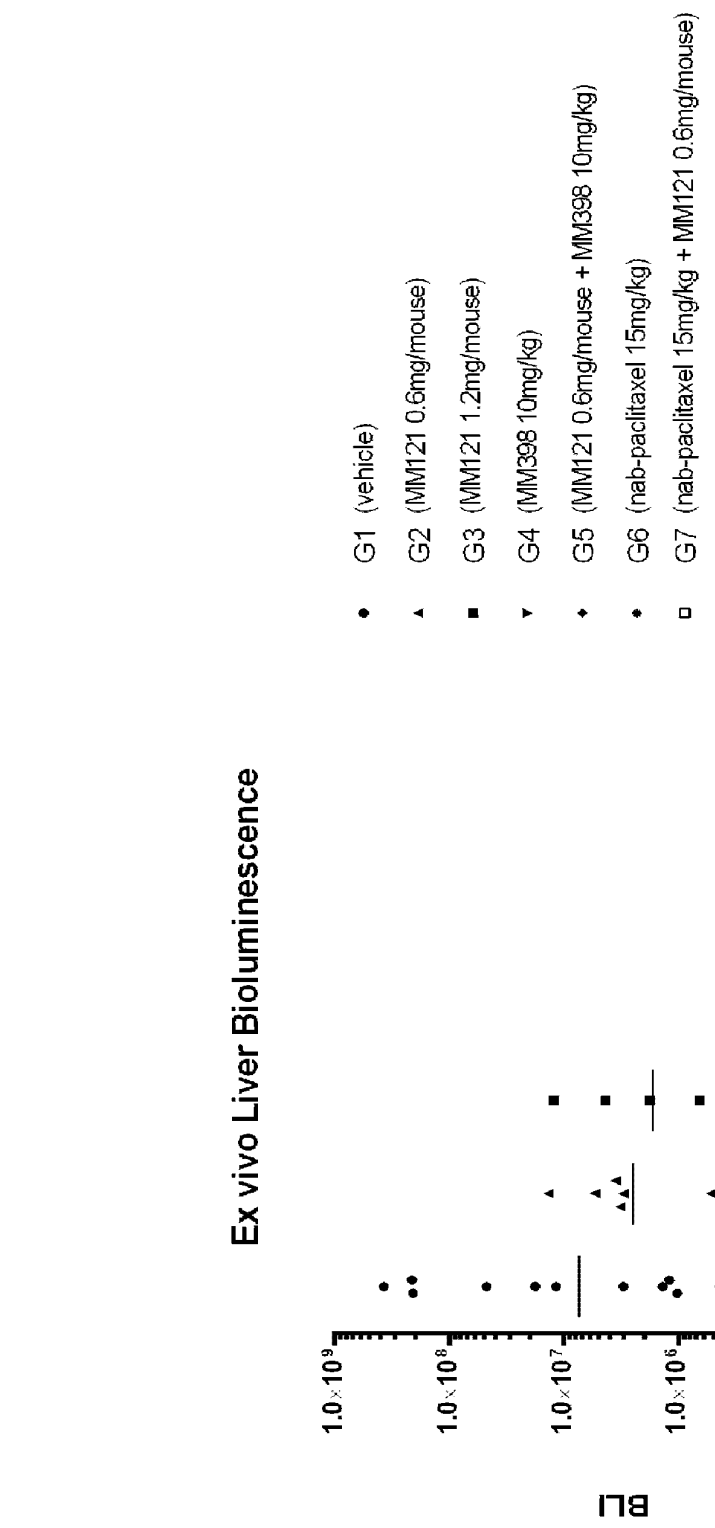

Ex vivo Liver Bioluminescence
G1 (vehicle)
G2 (MM121 0.6mg/mouse)
G3 (MM121 1.2mg/mouse)
G4 (MM398 10mg/kg)
G5 (MM121 0.6mg/mouse + MM398 10mg/kg)
G6 (nab-paclitaxel 15mg/kg)
G7 (nab-paclitaxel 15mg/kg + MM121 0.6mg/mouse)
BLI
1.0×10^9
1.0×10^8
1.0×10^7
1.0×10^6
1.0×10^5
1.0×10^4
G1
G2
G3
G4
G5
G6
G7
Treatment Groups

Fig. 8B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/30585

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/00; C12P 21/08 (2013.01)
USPC - 424/133.1, 424/139.1; 530/387.3, 530/387.9
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 39/00; C12P 21/08; A61K 39/395; C07K 16/00 (2013.01)
USPC - 424/133.1, 424/139.1; 530/387.3, 530/387.9; 424/130.1, 424/141.1; 530/387.1, 530/388.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8) - A61K 39/00; C12P 21/08; A61K 39/395; C07K 16/00 (2013.01) - see keyword below
USPC - 424/133.1, 424/139.1; 530/387.3, 530/387.9; 424/130.1, 424/141.1; 530/387.1, 530/388.1 - see keyword below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(USPT,PGPB,EPAB,JPAB); PatBase; Medline, Google: anti-ErbB3, HER3, antibody, EGFR, inhibitor, antagonist, gefitinib, erlotinib, pancreatic, cancer, malignant, neoplastic, tumor, 8B8, 1B4C3, 2D1DI2, GE-huMab-HER3, MED13379, AMG888, AV-203, Heavy chain, light chain, CDR, variable region, administer, oral, co-administer, composition, treat, MM-

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/0266584 A1 (SCHOEBERL et al.) 21 October 2010 (21.10.2010), para [0005], [0008], [0036], [0040], [0041], [0047], [0148], [0153], [0155], [0206], [0209], [0220], [0228], [0374], [0389], SEQ ID NO: 1 and SEQ ID NO: 2	1-2, 18-20/(1-2), 36/(1-2), 58-59, 74-77/(58-59), 90/(58-59), 112
A	KELLEY et al. Erlotinib in the treatment of advanced pancreatic cancer. Biologics. 2008, Vol.. 2(1), p. 83-95. Entire documentation, especially Abstract	1-2, 18-20/(1-2), 36/(1-2), 58-59, 74-77/(58-59), 90/(58-59), 112
A	MOHAMMED et al. The epidermal growth factor receptor inhibitor gefitinib prevents the progression of pancreatic lesions to carcinoma in a conditional LSL-KrasG12D/+ transgenic mouse model Cancer Prev Res (Phila). 2010, Vol. 3(11), p. 1417-26. Entire documentation, especially Abstract; pg 1421, col 2, lower para, and Fig 2; and pg 1424, col 2, top para	1-2, 18-20/(1-2), 36/(1-2), 58-59, 74-77/(58-59), 90/(58-59), 112
A	US 2011/0008327 A1 (CHENG et al) 13 January 2011 (13.01.2011), para [0221]	1-2, 18-20/(1-2), 36/(1-2), 58-59, 74-77/(58-59), 90/(58-59), 112
A	MATAR et al. Combined Epidermal Growth Factor Receptor Targeting with the Tyrosine Kinase Inhibitor Gefitinib (ZD1839) and the Monoclonal Antibody Cetuximab (IMC-C225): Superiority Over Single-Agent Receptor Targeting. Clin Cancer Res. 2004, Vol. 10(19), p. 6487-501. Entire documentation, especially Abstract	1-2, 18-20/(1-2), 36/(1-2), 58-59, 74-77/(58-59), 90/(58-59), 112

☐ 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
26 June 2013 (26.06.2013)	11 JUL 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/30585

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

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

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

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

3. ☒ Claims Nos.: 21-31, 41-57, 78-85, 95-111
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+, claims 1-20, 32-40, 58-77, 86-94, 112-121, drawn to methods of treating pancreatic cancer in a patient comprising: co-administering to the patient an anti-ErbB3 antibody and one or more additional therapeutic agents; and compositions thereof. The first invention (claims 1-2, 18-20/(1-2), 36/(1-2), 58-59, 74-77/(58-59), 90/(58-59)), is restricted to co-administering to the patient an anti-ErbB3 antibody and gefitinib (the first option for an EGFR inhibitor in claim 2), which will be searched without additional fee. Applicant is invited to elect additional therapeutic agents to be searched by paying an additional fee per additional therapeutic agent and clearly identifying the elected additional therapetuic agent(s) for co-administration. For example, applicant could elect EGFR inhibitor erlotinib (claims 1-2, 18-20/(1-2), 36/(1-2), 58-59, 74-77/(58-59), 90/(58-59), 112) with an additional fee; or applicant could elect a microtubule stabilizing agent (claims 9-12, 18-20/(9-12), 32-35, 66-69, 74-77/(66-69), 86-89, 117, 121) with an additional fee;

**********Continued in the the extra sheet**********

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

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

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-2, 18-20/(1-2), 36/(1-2), 58-59, 74-77/(58-59), 90/(58-59), 112, limited to gefitinib and erlotinib.

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

Remark on Protest

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

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

☒ No protest accompanied the payment of additional search fees.

Continuation of:
Boc No III (unity of invention is lacking)

(Continuation of Group I+) or applicant could elect EGFR inhibitor erlotinib and a nucleoside metabolic inhibitor [e.g. gemcitabine] (claims 1-2, 4-8, 18-20/(1-2, 4-8), 36/(1-2, 4-6), 37-40, 58-59, 61-65, 74-77(58-59, 61-65), 90/(58-59, 61-65), 91-94, 112, 113, 120) with 2X additional fees. The exact claims to be searched will depend on the election. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group will result in only the first claimed invention to be searched.

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Groups I+ share the technical features of a composition for the treatment of pancreatic cancer comprising an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents, and a method of treating pancreatic cancer in a patient comprising: co-administering to the patient an effective amount of each of an anti-ErbB3 antibody and one or more additional therapeutic agents. Some claims within Group I+ (partial) also share the technical features of wherein the one or more additional therapeutic agents comprise an EGFR inhibitor. Some claims within Group I+ (partial) also share the technical features of an anti-ErbB3 antibody having the heavy chain sequence set forth in SEQ ID NO:2 and the light chain sequence set forth in SEQ ID NO:4; or an anti-ErbB3 antibody comprises CDRH1, CDRH2, and CDRH3 sequences comprising the amino acid sequences set forth in SEQ ID NO: 5 (CDRH1), SEQ ID NO: 6 (CDRH2) and SEQ ID NO: 7 (CDRH3), and further comprises CDRL1, CDRL2, and CDRL3 sequences comprising the amino acid sequences set forth in SEQ ID NO:8 (CDRL1) SEQ ID NO: 9 (CDRL2) and SEQ ID NO: 10 (CDRL3); and wherein anti-ErbB3 antibody is selected from 8B8, lB4C3, 2DIDI2, GE-huMab-HER3, MEDI3379, AMG888 and AV-203.

However, these shared technical features do not represent a contribution over prior art as being anticipated by US 2010/0266584 A1 to Schoeberl et al. (hereinafter 'Schoeberl') as follows:

Schoeberl discloses a method of treating pancreatic cancer in a patient (para [0040], [0148]) comprising co-administering to the patient an effective amount of each of an anti-ErbB3 antibody (para [0005], [0040]- [0041], [0148]), and one or more additional therapeutic agents, wherein the one of the one or more additional therapeutic agents comprise an EGFR inhibitor (claims 2 and 3; para ([0041]-[0058] - 'binding to ErbB1 ...and the inhibition of such binding by cetuximab').
Schoeberl further discloses an anti-ErbB3 antibody heavy chain and light chain variable sequences (para [0047] - 'an anti-ErbB3 antibody (Ab #6)'; para [0008] - 'a heavy chain variable region (V.sub.H) ... SEQ ID NO:1, ... a light chain variable region (V.sub.L) ... SEQ ID NO:2'; para [0389] - 'mapping of Ab #6 is performed ... the V.sub.H region (SEQ ID NO: 1) and the V.sub.L region (SEQ ID NO: 2)', wherein SEQ ID NO: 1 is 100% identical to the claimed SEQ ID NO: 2, and comprising a region between nucleotides 30-35, that is 100% identical to the claimed SEQ ID NO: 5 (CDRH1), a region between nucleotides 50-66, that is 100% identical to the claimed SEQ ID NO: 6 (CDRH2), and a region between nucleotides 99-108, that is 100% identical to the claimed SEQ ID NO: 7 (CDRH3); and wherein SEQ ID NO: 2 is 100% identical to the claimed SEQ ID NO: 4, and comprising a region between nucleotides 23-36, that is 100% identical to the claimed SEQ ID NO: 8 (CDRL1), a region between nucleotides 52-58, that is 100% identical to the claimed SEQ ID NO: 9 (CDRL2), and a region between nucleotides 91-101, that is 100% identical to the claimed SEQ ID NO: 10 (CDRL3)), and different anti-ErbB3 including anti -ErbB3 antibody selected from the group consisting of 2DIDI2, and AMG888 (Table I - Anti-ErbB3 antibodies Ab #14 described herein which bind different 1B4C3; 2D1D12 (U3 Pharma AG) epitopes U3-1287/AMG888 (U3 Pharma/Amgen)').

Without a shared special technical feature, the inventions lack unity with one another.

Therefore, inventions of Groups I+lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note re item 4: Claims 21-31, 41-57, 78-85, 95-111 are not drafted in accordance with the second and third sentences of Rule 6.4 (a). These claims are improper multiple dependent claims.

Note:

I) Claims 11 and 68 are objected to as lacking a proper antecedent basis for the "the one or more additional therapeutic agents comprise a microtubule stabilizing agent" limitation. It is assumed that claim 11 depends upon claim 9 and claim 68 depends upon claim 66 (Specification: pg 4, ln 9-10 'a microtubule stabilizing agent, e.g., a taxane such as eribulin, ... nab-paclitaxel'). For the purposes of this ISR, claims 11 and 68 are construed as follows:

11. The method of claim 9, wherein the one or more additional therapeutic agents is nab-paclitaxel.

68. The composition of claim 66, wherein the one or more additional therapeutic agents is nab- paclitaxel.

II) Claims 20 and 77 are objected to as using an improper Markush group. For the purposes of this ISR, the term 'selected from' in each claim is construed as 'selected from the group consisting of'.

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— 国際調査報告（条約第 21 条(3)）

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(54) **Title**: LOW-DOSE ANTITUMOR AGENT INCLUDING IRINOTECAN HYDROCHLORIDE HYDRATE

(54) 発明の名称 : 低用量イリノテカン塩酸塩水和物を含有する抗腫瘍剤

(57) **Abstract**: Provided is a novel combination therapy using an FTD/TPI combination drug which exhibits remarkable antitumor effects, and few side effects. This antitumor agent is characterized in that the FTD/TPI combination drug is administered, using a reduced amount of FTD, at a dosage in the range 35-70 mg/m^2/day, and CPT-11 is administered at a dosage in the range 45-144 mg/m^2/day.

(57) 要約:　顕著な抗腫瘍効果を示し、副作用の少ないＦＴＤ・ＴＰＩ配合剤の新規な併用療法の提供。ＦＴＤ・ＴＰＩ配合剤がＦＴＤ換算量で３５～７０ｍｇ／ｍ^2／ｄａｙ投与され、ＣＰＴ－１１が４５～１４４ｍｇ／ｍ^2／ｄａｙ投与されることを特徴とする抗腫瘍剤。

明　細　書

発明の名称 ：

低用量イリノテカン塩酸塩水和物を含有する抗腫瘍剤

技術分野

[0001] 本発明は、トリフルリジンとチピラシル塩酸塩の配合剤、及びイリノテカン塩酸塩水和物を併用してなる抗腫瘍剤、及びイリノテカン塩酸塩水和物の抗腫瘍効果増強剤に関する。

背景技術

[0002] トリフルリジン（別名：α，α，α－トリフルオロチミジン。以下、「ＦＴＤ」とも称す）は、チミジレート生成阻害作用とＤＮＡへの取り込みによるＤＮＡ合成阻害作用により抗腫瘍効果を発揮する。一方、チピラシル塩酸塩（化学名：５－クロロ－６－［（２－イミノピロリジン－１－イル）メチル］ピリミジン－２，４（１Ｈ，３Ｈ）－ジオン塩酸塩。以下、「ＴＰＩ」とも称す）は、チミジンホスホリラーゼ阻害作用を有する。ＴＰＩがチミジンホスホリラーゼによるＦＴＤの生体内での分解を抑制することにより、ＦＴＤの抗腫瘍効果が増強されることが知られている（特許文献１）。現在、ＦＴＤとＴＰＩをモル比１：０．５で含有する抗腫瘍剤（以下、「ＦＴＤ・ＴＰＩ配合剤」とも称す）は、結腸直腸癌等の固形癌の治療剤として開発中である（非特許文献１及び２）。

[0003] また、イリノテカン塩酸塩水和物（以下、「ＣＰＴ－１１」とも称す）は、ＳＮ－３８を活性代謝物とするカンプトテシン誘導体であり、トポイソメラーゼＩを阻害することによりＤＮＡの合成及び転写を抑制し、抗腫瘍効果を発揮する。ＣＰＴ－１１は、小細胞肺癌、非小細胞肺癌、子宮頸癌、卵巣癌、胃癌、結腸・直腸癌、乳癌、有棘細胞癌、悪性リンパ腫などの幅広い癌種の治療剤として臨床で用いられている（非特許文献３）。

さらに、結腸直腸癌の細胞株に対してＦＴＤとＳＮ－３８を作用させたところ、相乗的な細胞毒性が確認されたことから、ＦＴＤ・ＴＰＩ配合剤とＣ

ＰＴ－１１の併用療法が期待されている（非特許文献４）。

先行技術文献

特許文献

[0004] 特許文献1：国際公開第９６／３０３４６号

非特許文献

[0005] 非特許文献1：Ｉｎｖｅｓｔ　Ｎｅｗ　Ｄｒｕｇｓ　２６（５）：４４５－５４，２００８．

非特許文献2：Ｌａｎｃｅｔ　Ｏｎｃｏｌ．１３（１０）：９９３－１００１，２０１２．

非特許文献3：Ｏｎｃｏｌｏｇｉｓｔ．６（１）：６６－８０，２００１．

非特許文献4：Ｅｕｒ　Ｊ　Ｃａｎｃｅｒ．４３（１）：１７５－８３，２００７．

発明の概要

発明が解決しようとする課題

[0006] 本発明は、顕著な抗腫瘍効果を示し、副作用の少ないＦＴＤ・ＴＰＩ配合剤の新規な固形癌の併用療法を提供することを課題とする。

課題を解決するための手段

[0007] 本発明者はこのような現状に鑑み、後述する比較例の通り、各薬剤において既に効果が報告されている投与量に基づき、結腸直腸癌患者に対して、ＦＴＤ・ＴＰＩ配合剤を７０ｍｇ／ｍ^2／ｄａｙで５日間投与した後２日間休薬することを２回行い、その後２週間休薬し、ＣＰＴ－１１を１５０ｍｇ／ｍ^2／ｄａｙで２週間に１回投与する２８日間のサイクルを繰り返す併用療法を行ったところ、好中球減少、下痢及び体重減少等の副作用が強く現れたため、ＣＰＴ－１１は予定量の３０％程度しか投与できなかった。本発明者らは、副作用の発生を抑え、予定量を投与できる投与スケジュールについて研究を重ねた結果、固形癌患者に対して、ＦＴＤ・ＴＰＩ配合剤をＦＴＤ換算量で３５～７０ｍｇ／ｍ^2／ｄａｙ投与し、ＣＰＴ－１１を４５～１４４ｍｇ／

m^2／ｄａｙ投与する併用療法が、副作用の発生を抑え且つ優れた抗腫瘍効果を奏することを見出した。

[0008] すなわち本発明は、次の〔１〕～〔３２〕を提供するものである。

[0009] 〔１〕トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／m^2／ｄａｙ投与され、イリノテカン塩酸塩水和物が４５～１４４ｍｇ／m^2／ｄａｙ投与されることを特徴とする固形癌に対する抗腫瘍剤。

〔２〕トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤が、トリフルリジン換算量で７０ｍｇ／m^2／ｄａｙで投与される〔１〕記載の抗腫瘍剤。

〔３〕イリノテカン塩酸塩水和物が、７５～１２０ｍｇ／m^2／ｄａｙ投与される〔１〕又は〔２〕記載の抗腫瘍剤。

〔４〕固形癌が、結腸直腸癌、肺癌、乳癌、膵癌又は胃癌である〔１〕～〔３〕のいずれかに記載の抗腫瘍剤。

〔５〕トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤を第１日目から第５日目までと第８日目から第１２日目まで投与し、ＣＰＴ－１１を第１日目と第１５日目に投与する２８日間１サイクルの投与スケジュールを１回又は２回以上繰り返すことを特徴とする〔１〕～〔４〕のいずれかに記載の抗腫瘍剤。

〔６〕固形癌患者に対するイリノテカン塩酸塩水和物の抗腫瘍効果を増強するための、トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤からなる抗腫瘍効果増強剤であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／m^2／ｄａｙ投与され、イリノテカン塩酸塩水和物が４５～１４４ｍｇ／m^2／ｄａｙ投与されることを特徴とする抗腫瘍効果増強剤。

〔７〕イリノテカン塩酸塩水和物を投与された固形癌患者を治療するための、トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合

剤からなる抗腫瘍剤であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／ｍ^2／ｄａｙ投与され、

イリノテカン塩酸塩水和物が４５～１４４ｍｇ／ｍ^2／ｄａｙ投与されることを特徴とする抗腫瘍剤。

〔８〕トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤を含む抗腫瘍剤と使用説明書を含むキット製剤であって、

使用説明書には、固形癌患者に対して、トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／ｍ^2／ｄａｙ投与され、

イリノテカン塩酸塩水和物が４５～１４４ｍｇ／ｍ^2／ｄａｙ投与されることが記載されていることを特徴とするキット製剤。

〔９〕固形癌治療のためのトリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／ｍ^2／ｄａｙ投与され、イリノテカン塩酸塩水和物が４５～１４４ｍｇ／ｍ^2／ｄａｙ投与されることを特徴とする配合剤。

〔10〕トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤が、トリフルリジン換算量で７０ｍｇ／ｍ^2／ｄａｙで投与される〔9〕記載の配合剤。

〔11〕イリノテカン塩酸塩水和物が、７５～１２０ｍｇ／ｍ^2／ｄａｙ投与される〔９〕又は〔１０〕記載の配合剤。

〔12〕固形癌が、結腸直腸癌、肺癌、乳癌、膵癌又は胃癌である〔９〕～〔１１〕のいずれかに記載の配合剤。

〔13〕トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤を第１日目から第５日目までと第８日目から第１２日目まで投与し、ＣＰＴ－１１を第１日目と第１５日目に投与する２８日間１サイクルの投与

スケジュールを１回又は２回以上繰り返すことを特徴とする〔９〕～〔１２〕いずれかに記載の配合剤。

〔14〕固形癌患者に対するイリノテカン塩酸塩水和物の抗腫瘍効果を増強するための、トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／m^2／ｄａｙ投与され、イリノテカン塩酸塩水和物が４５～１４４ｍｇ／m^2／ｄａｙ投与されることを特徴とする配合剤。

〔15〕イリノテカン塩酸塩水和物を投与された固形癌患者を治療するための、トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／m^2／ｄａｙ投与され、

イリノテカン塩酸塩水和物が４５～１４４ｍｇ／m^2／ｄａｙ投与されることを特徴とする配合剤。

〔16〕トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する合剤の固形癌に対する抗腫瘍剤製造のための使用であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／m^2／ｄａｙ投与され、イリノテカン塩酸塩水和物が４５～１４４ｍｇ／m^2／ｄａｙ投与されることを特徴とする使用。

〔17〕トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤が、トリフルリジン換算量で７０ｍｇ／m^2／ｄａｙで投与される〔１６〕記載の使用。

〔18〕イリノテカン塩酸塩水和物が、７５～１２０ｍｇ／m^2／ｄａｙ投与される〔１６〕又は〔１７〕記載の使用。

〔19〕固形癌が、結腸直腸癌、肺癌、乳癌、膵癌又は胃癌である〔１６〕～

〔１８〕のいずれかに記載の使用。

〔20〕トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤を第１日目から第５日目までと第８日目から第１２日目まで投与し、ＣＰＴ－１１を第１日目と第１５日目に投与する２８日間１サイクルの投与スケジュールを１回又は２回以上繰り返すことを特徴とする〔１６〕～〔１９〕のいずれかに記載の使用。

〔21〕固形癌患者に対するイリノテカン塩酸塩水和物の抗腫瘍効果を増強するための、トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤からなる抗腫瘍効果増強剤製造のための使用であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／m^2／ｄａｙ投与され、イリノテカン塩酸塩水和物が４５～１４４ｍｇ／m^2／ｄａｙ投与されることを特徴とする使用。

〔22〕イリノテカン塩酸塩水和物を投与された固形癌患者を治療するための、トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤からなる抗腫瘍剤製造のための使用であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／m^2／ｄａｙ投与され、

イリノテカン塩酸塩水和物が４５～１４４ｍｇ／m^2／ｄａｙ投与されることを特徴とする使用。

〔23〕固形癌患者に対してトリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／m^2／ｄａｙ投与され、イリノテカン塩酸塩水和物が４５～１４４ｍｇ／m^2／ｄａｙ投与されることを特徴とする固形癌の治療方法。

〔24〕トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤が、トリフルリジン換算量で７０ｍｇ／m^2／ｄａｙで投与される〔２３〕記載の方法。

〔25〕イリノテカン塩酸塩水和物が、７５～１２０ｍｇ／m^2／ｄａｙ投与さ

れる〔２３〕又は〔２４〕記載の方法。

〔26〕固形癌が、結腸直腸癌、肺癌、乳癌、膵癌又は胃癌である〔２３〕～〔２５〕のいずれかに記載の方法。

〔27〕トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤を第１日目から第５日目までと第８日目から第１２日目まで投与し、ＣＰＴ－１１を第１日目と第１５日目に投与する２８日間１サイクルの投与スケジュールを１回又は２回以上繰り返すことを特徴とする〔２３〕～〔２６〕のいずれかに記載の方法。

〔28〕固形癌患者に対するイリノテカン塩酸塩水和物の抗腫瘍効果を増強するための方法であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／m^2／ｄａｙ投与され、イリノテカン塩酸塩水和物が４５～１２０ｍｇ／m^2／ｄａｙ投与されることを特徴とする方法。

〔29〕イリノテカン塩酸塩水和物を投与された固形癌患者を治療するための方法であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／m^2／ｄａｙ投与され、

イリノテカン塩酸塩水和物が４５～１２０ｍｇ／m^2／ｄａｙ投与されることを特徴とする方法。

〔30〕トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤とイリノテカン塩酸塩水和物を併用投与することを特徴とする固形癌に対する抗腫瘍剤であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤の投与量が、単独療法における推奨投与量の５０％～１００％であり、

イリノテカン塩酸塩水和物の投与量が、単独療法における推奨投与量の２５％～８０％である抗腫瘍剤。

〔31〕トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する

配合剤の投与量が、単独療法における推奨投与量の１００％である〔30〕記載の抗腫瘍剤。

〔32〕イリノテカン塩酸塩水和物の投与量が、単独療法における推奨投与量の５０％～７０％である〔30〕又は〔31〕記載の抗腫瘍剤。

発明の効果

[0010] 本発明の抗腫瘍剤によれば、副作用の発症を抑えつつ、高い抗腫瘍効果を奏する癌治療を行うことが可能であり、よって患者の長期間の生存をもたらす。

図面の簡単な説明

[0011] [図1]ＦＴＤ・ＴＰＩ配合剤　１５０ｍｇ／ｋｇ／ｄａｙ及びＣＰＴ－１１　５０ｍｇ／ｋｇ／ｄａｙの併用による抗腫瘍効果を示す図である。

[図2]ＦＴＤ・ＴＰＩ配合剤　１５０ｍｇ／ｋｇ／ｄａｙ及びＣＰＴ－１１　２５ｍｇ／ｋｇ／ｄａｙの併用による抗腫瘍効果を示す図である。

[図3]ＦＴＤ・ＴＰＩ配合剤　１５０ｍｇ／ｋｇ／ｄａｙ及びＣＰＴ－１１　１０ｍｇ／ｋｇ／ｄａｙの併用による抗腫瘍効果を示す図である。

[図4]ＦＴＤ・ＴＰＩ配合剤　７５ｍｇ／ｋｇ／ｄａｙ及びＣＰＴ－１１　５０ｍｇ／ｋｇ／ｄａｙの併用による抗腫瘍効果を示す図である。

[図5]ＦＴＤ・ＴＰＩ配合剤　７５ｍｇ／ｋｇ／ｄａｙ及びＣＰＴ－１１　２５ｍｇ／ｋｇ／ｄａｙの併用による抗腫瘍効果を示す図である。

[図6]ＦＴＤ・ＴＰＩ配合剤　７５ｍｇ／ｋｇ／ｄａｙ及びＣＰＴ－１１　１０ｍｇ／ｋｇ／ｄａｙの併用による抗腫瘍効果を示す図である。

[図7]ＦＴＤ・ＴＰＩ配合剤　１５０ｍｇ／ｋｇ／ｄａｙ及びＣＰＴ－１１　５０、７０又は１００ｍｇ／ｋｇ／ｄａｙの各併用投与群におけるＤａｙ７０での生存率を示す図である。

発明を実施するための形態

[0012] 本発明のＦＴＤ及びＴＰＩはそれぞれ公知の化合物であり、例えば、国際公開第９６／３０３４６号パンフレットに記載の方法に準じて合成することができる。また、ＦＴＤ及びＴＰＩをモル比１：０．５で含有する配合剤も

公知である（非特許文献１及び２）。

本発明のＣＰＴ－１１は公知の化合物であり、特許第３００４０７７号公報に記載の方法に準じて合成することができる。また、カンプト（登録商標）（株式会社ヤクルト本社）などの市販品を用いても良い。

[0013] 本発明の抗腫瘍剤の投与スケジュールは、本発明の効果を奏する限り特に制限はないが、ＦＴＤ及びＴＰＩをモル比１：０．５で含有する配合剤を第１日目から第５日目までと第８日目から第１２日目まで投与し、ＣＰＴ－１１を第１日目と第１５日目に投与する２８日間１サイクルの投与スケジュールを１回又は２回以上繰り返して実施することが好ましい。

[0014] 後述の参考例及び実施例のとおり、マウスに対する単独療法における推奨投与量の５０％～１００％のＦＴＤ・ＴＰＩ配合剤と、マウスに対する単独療法における推奨投与量の２５％～８０％のＣＰＴ－１１を、マウスに併用投与した場合、優れた抗腫瘍効果と副作用の抑制を達成できた。したがって、本発明におけるＦＴＤ・ＴＰＩ配合剤の投与量は、ヒトに対する単独療法における推奨投与量の５０％～１００％であり、抗腫瘍効果と副作用のバランスの観点から１００％が特に好ましい。ＣＰＴ－１１の投与量は、ヒトに対する単独療法における推奨投与量の２５％～８０％であり、抗腫瘍効果と副作用のバランスの観点から、４０％～８０％が好ましく、５０％～８０％がより好ましく、５０％～７０％が特に好ましい。

すなわち、ＦＴＤ・ＴＰＩ配合剤のヒトに対する単独療法における推奨投与量は７０ｍｇ／ｍ^2／ｄａｙであることから、ＦＴＤの投与量は３５～７０ｍｇ／ｍ^2／ｄａｙであり、抗腫瘍効果と副作用のバランスの観点から、７０ｍｇ／ｍ^2／ｄａｙが特に好ましい。

ＣＰＴ－１１のヒトに対する単独療法における推奨投与量は、投与スケジュールによって異なるが、例えば、２週間毎の投与の場合、１５０～１８０ｍｇ／ｍ^2／ｄａｙである。よって、その推奨投与量が１８０ｍｇ／ｍ^2／ｄａｙのとき（例えば、消化器癌、肺癌、乳癌、子宮頸癌又は卵巣癌等が挙げられ、結腸直腸癌及び膵癌が好ましい）は、本発明のＣＰＴ－１１の投与量は

４５～１４４ｍｇ／m^2／ｄａｙであり、抗腫瘍効果と副作用のバランスの観点から、７２～１４４ｍｇ／m^2／ｄａｙが好ましく、９０～１４４ｍｇ／m^2／ｄａｙがより好ましく、９０～１２６ｍｇ／m^2／ｄａｙが特に好ましい。また、その推奨投与量が１５０ｍｇ／m^2／ｄａｙのとき（例えば、消化器癌、肺癌、乳癌、子宮頸癌又は卵巣癌等が挙げられ、子宮頸癌、卵巣癌、胃癌及び結腸直腸癌が好ましい）は、本発明のＣＰＴ－１１の投与量は３７．５～１２０ｍｇ／m^2／ｄａｙであり、抗腫瘍効果と副作用のバランスの観点から、６０～１２０ｍｇ／m^2／ｄａｙが好ましく、７５～１２０ｍｇ／m^2／ｄａｙがより好ましく、７５～１０５ｍｇ／m^2／ｄａｙが特に好ましい。

また、１週間毎の投与の場合、ＣＰＴ－１１のヒトに対する単独療法における推奨投与量は１００～１２５ｍｇ／m^2／ｄａｙである。よって、その推奨投与量が１００ｍｇ／m^2／ｄａｙのとき（例えば、消化器癌、肺癌、乳癌、子宮頸癌又は卵巣癌等が挙げられ、小細胞肺癌、非小細胞肺癌、乳癌 、子宮頸癌、卵巣癌、胃癌及び結腸直腸癌が好ましい）は、本発明のＣＰＴ－１１の投与量は２５～８０ｍｇ／m^2／ｄａｙであり、抗腫瘍効果と副作用のバランスの観点から、４０～８０ｍｇ／m^2／ｄａｙが好ましく、５０～８０ｍｇ／m^2／ｄａｙがより好ましく、５０～７０ｍｇ／m^2／ｄａｙが特に好ましい。また、その推奨投与量が１２５ｍｇ／m^2／ｄａｙのとき（例えば、消化器癌、肺癌、乳癌、子宮頸癌又は卵巣癌等が挙げられ、結腸直腸癌が好ましい）は、本発明のＣＰＴ－１１の投与量は３１．２５～１００ｍｇ／m^2／ｄａｙであり、抗腫瘍効果と副作用のバランスの観点から、５０～１００ｍｇ／m^2／ｄａｙが好ましく、６２．５～１００ｍｇ／m^2／ｄａｙがより好ましく、６２．５～８７．５ｍｇ／m^2／ｄａｙが特に好ましい。

また、３週間毎の投与の場合、ＣＰＴ－１１のヒトに対する単独療法における推奨投与量は３５０ｍｇ／m^2／ｄａｙである（例えば、消化器癌、肺癌、乳癌、子宮頸癌又は卵巣癌等が挙げられ、結腸直腸癌が好ましい）。よって、本発明のＣＰＴ－１１の投与量は８７．５～２８０ｍｇ／m^2／ｄａｙであり、抗腫瘍効果と副作用のバランスの観点から、１４０～２８０ｍｇ／m^2

／ｄａｙが好ましく、１７５～２８０ｍｇ／m^2／ｄａｙがより好ましく、１７５～２４５ｍｇ／m^2／ｄａｙが特に好ましい。

[0015] 本発明の抗腫瘍剤の対象は固形癌であり、具体的には、頭頸部癌、消化器癌（食道癌、胃癌、十二指腸癌、肝臓癌、胆道癌（胆嚢・胆管癌など）、膵臓癌、小腸癌、大腸癌（結腸直腸癌、結腸癌、直腸癌など）など）、肺癌、乳癌、卵巣癌、子宮癌（子宮頚癌、子宮体癌など）、腎癌、膀胱癌、前立腺癌等が挙げられる。このうち、抗腫瘍効果と副作用の観点から、消化器癌、肺癌、乳癌、子宮頸癌又は卵巣癌が好ましく、結腸直腸癌、肺癌、乳癌、膵癌又は胃癌がより好ましく、結腸直腸癌及び胃癌がより好ましく、結腸直腸癌が特に好ましい。なお、ここで固形癌には、原発巣のみならず、他の臓器（肝臓など）に転移した固形癌由来の腫瘍をも含む。また、本発明の抗腫瘍剤は、腫瘍を外科的に摘出した後に再発防止のために行われる術後補助化学療法に用いるものであってもよい。

[0016] 各有効成分で投与手段や投与スケジュールが異なり、全ての有効成分を一つの剤形にまとめて製剤化することはできないため、本発明の抗腫瘍剤は各有効成分を複数の剤形に分けて製剤化する。ＦＴＤ及びＴＰＩは配合剤として、ＣＰＴ－１１は単剤として製剤化することが好ましい。

[0017] また、本発明の投与量によって各有効成分が投与される限り、各製剤を併用投与に適した１個のパッケージにまとめて製造販売してもよく、また各製剤を別個のパッケージに分けて製造販売してもよい。

[0018] 本発明の抗腫瘍剤の投与形態としては特に制限は無く、治療目的に応じて適宜選択でき、具体的には経口剤（錠剤、被覆錠剤、散剤、顆粒剤、カプセル剤、液剤など）、注射剤、坐剤、貼付剤、軟膏剤等が例示できる。ＦＴＤ及びＴＰＩの配合剤は経口剤が、ＣＰＴ－１１は注射剤が好ましい。

[0019] 本発明における抗腫瘍剤は、その投与形態に応じて、薬学的に許容される担体を用いて、通常公知の方法により調製することができる。斯かる担体としては、通常の薬剤に汎用される各種のもの、例えば賦形剤、結合剤、崩壊剤、滑沢剤、希釈剤、溶解補助剤、懸濁化剤、等張化剤、ｐＨ調整剤、緩衝

剤、安定化剤、着色剤、矯味剤、矯臭剤等を例示できる。

[0020] 本発明はまた、上記投与量に基づきＦＴＤ・ＴＰＩ配合剤及びＣＰＴ－１１が投与されることを特徴とする、固形癌患者（特に、結腸直腸癌患者）に対するＣＰＴ－１１の抗腫瘍効果を増強するためのＦＴＤ・ＴＰＩ配合剤を含む抗腫瘍効果増強剤に関する。当該抗腫瘍効果増強剤は、上記抗腫瘍剤の製剤形態を有する。

[0021] 本発明はまた、上記投与量に基づきＦＴＤ・ＴＰＩ配合剤及びＣＰＴ－１１が投与されることを特徴とする、ＣＰＴ－１１を投与された固形癌患者（特に、結腸直腸癌患者）を治療するためのＦＴＤ・ＴＰＩ配合剤を含む抗腫瘍剤に関する。当該抗腫瘍剤は、上記の製剤形態を有する。

[0022] 本発明はまた、固形癌患者（特に、結腸直腸癌患者）に対する、ＦＴＤ・ＴＰＩ配合剤、及び上記投与量に基づきＦＴＤ・ＴＰＩ配合剤及びＣＰＴ－１１が投与されることを記載した使用説明書を含むキット製剤に関する。ここで「使用説明書」とは、上記投与量が記載されたものであればよく、法的拘束力の有無を問わないが、上記投与量が推奨されているものが好ましい。具体的には、添付文書、パンフレット等が例示される。また、使用説明書を含むキット製剤とは、キット製剤のパッケージに使用説明書が印刷・添付されているものであっても、キット製剤のパッケージに抗腫瘍剤とともに使用説明書が同封されているものであってもよい。

実施例 1

[0023] 次に実施例を挙げて本発明をさらに詳細に説明する。

[0024] 参考例

ヒト大腸癌株（ＫＭ２０Ｃ）の培養細胞（１×１０^{7}ｃｅｌｌｓ／マウス）を生後５～６週齢のＢＡＬＢ／ｃＡ　Ｊｃｌ－ｎｕマウスの腹腔内に移植し、各群の平均体重が均等になるように各群にマウスを割り付け、群分け（ｎ＝１０）を実施した日をＤａｙ　０とした。

ＦＴＤ・ＴＰＩ配合剤（ＦＴＤとＴＰＩのモル比１：０．５の混合物）は、ＦＴＤとして７５、１００、１５０、３００及び４５０ｍｇ／ｋｇ／ｄａ

ｙとなるように調製した。イリノテカン塩酸塩水和物（ＣＰＴ－１１：カンプト注（登録商標）、株式会社ヤクルト本社）は、１１１ｍｇ／ｋｇ／ｄａｙで死亡例が報告されていることから（基礎と臨床、（１９９０）、Ｖｏｌ．２４、Ｎｏ．１４、７～１７）、イリノテカン塩酸塩水和物として８０及び１００ｍｇ／ｋｇ／ｄａｙとなるように調製した。薬剤の投与はＤａｙ３から開始し、ＦＴＤ・ＴＰＩ配合剤は５日間連日経口投与・２日間休薬を６週間行い、ＣＰＴ－１１は週に１回の尾静脈から投与を６週間行った。

抗腫瘍効果の指標として、各群のマウスの生存数を確認し、各群の生存期間を比較した。結果を表１に示す。

[0025]

[表1]

Drug	Dose (mg/kg/day)	Treatment[a)]	No. of animals	Survival time (day) Mean ± SD	ILS[b)] (%)
Control	–	–	10	40.0 ± 4.3	-
FTD·TPI	75	5-days' oral administration with 2-days' rest (b.i.d)	10	50.0 ± 9.1	25.0
FTD·TPI	100	5-days' oral administration with 2-days' rest (b.i.d)	10	75.8 ± 42.6	89.5
FTD·TPI	150	5-days' oral administration with 2-days' rest (b.i.d)	10	125.7 ± 64.8	214.3
FTD·TPI	300	5-days' oral administration with 2-days' rest (b.i.d)	10	75.6 ± 17.5	89.0
FTD·TPI	450	5-days' oral administration with 2-days' rest (b.i.d)	10	54.1 ± 18.3	35.3
CTP-11	80	i.v., weekly	10	61.6 ± 12.6	54.0
CTP-11	100	i.v., weekly	10	72.5 ± 12.3	81.3

a) : Drugs were given for 6 weeks from Day 3.
b) : ILS means increase in life span.
ILS(%) = [(mean survival time of treatment group) / (mean survival time of control group)−1]× 100

[0026] 表１に記載のように、マウスでは、ＣＰＴ－１１は１００ｍｇ／ｋｇ／ｄａｙの群で生存期間が長かったことから、マウスにおけるＣＰＴ－１１の推

奨投与量（ＲＤ）はイリノテカン塩酸塩水和物として１００ｍｇ／ｋｇ／ｄａｙである。したがって、マウスにおける１００ｍｇ／ｋｇ／ｄａｙが、ヒトにおけるＲＤ１５０～１８０ｍｇ／ｍ^2／ｄａｙに相当する。

[0027] ＦＴＤ・ＴＰＩ配合剤では、ＦＴＤ換算量で１５０ｍｇ／ｋｇ／ｄａｙの群で生存期間が長かったことから、マウスにおけるＦＴＤ・ＴＰＩ配合剤のＲＤはＦＴＤ換算量で１５０ｍｇ／ｋｇ／ｄａｙである。したがって、マウスにおける１５０ｍｇ／ｋｇ／ｄａｙ（ＦＴＤ換算量）が、ヒトにおけるＲＤ７０ｍｇ／ｍ^2／ｄａｙ（ＦＴＤ換算量）に相当する。

[0028] 実施例１

ヒト大腸癌株（ＫＭ２０Ｃ）を生後５～６週齢のＢＡＬＢ／ｃＡ　Ｊｃｌ－ｎｕマウスの右側胸部に移植した。腫瘍移植後に腫瘍の長径（ｍｍ）および短径（ｍｍ）を測定し、腫瘍体積（ｔｕｍｏｒ　ｖｏｌｕｍｅ：ＴＶ）を算出後、各群の平均ＴＶが均等になるように各群にマウスを割り付け、群分け（ｎ＝６）を実施した日をＤａｙ０とした。

ＦＴＤ・ＴＰＩ配合剤（ＦＴＤとＴＰＩのモル比１：０．５の混合物）は、ＦＴＤとして７５及び１５０ｍｇ／ｋｇ／ｄａｙとなるように調製した。ＣＰＴ－１１（カンプト（登録商標）注、株式会社ヤクルト本社）は、イリノテカン塩酸塩水和物として１０、２５及び５０ｍｇ／ｋｇ／ｄａｙとなるように調製した。ＦＴＤ・ＴＰＩ配合剤はＤａｙ１－１４に連日経口投与し、ＣＰＴ－１１はＤａｙ１及びＤａｙ８に尾静脈から投与した。併用投与群は、単剤投与群と同じ投与量及び投与スケジュールでＦＴＤ・ＴＰＩ配合剤とＣＰＴ－１１を投与した。各薬剤投与群の一覧を表２に示した。

抗腫瘍効果の指標として、各群のＤａｙ４、８、１１、１５、１８及び２２のＴＶを算出し、下式によりＤａｙ０に対する相対腫瘍体積（ｒｅｌａｔｉｖｅ　ｔｕｍｏｒ　ｖｏｌｕｍｅ：ＲＴＶ）を求めてプロットし、無処置群（ｃｏｎｔｒｏｌ）、ＦＴＤ・ＴＰＩ配合剤投与群、ＣＰＴ－１１投与群及びＦＴＤ・ＴＰＩ配合剤とＣＰＴ－１１併用投与群のＲＴＶの経日的推移を比較した。

[0029] ＴＶ（ｍｍ^3）＝（長径×短径^2）／２

ＲＴＶ＝（Ｄａｙ２８におけるＴＶ）／（Ｄａｙ０におけるＴＶ）

[0030] [表2]

Drug	Dose (mg/kg/day)	Treatment
FTD·TPI	75 150	Day 1-14, p.o.
CPT-11	10 25 50	Day 1 and 8, i.v.
FTD·TPI+CPT-11	75+10 75+25 75+50 150+10 150+25 150+50	Day 1-14, p.o. (FTD·TPI) Day 1 and 8, i.v. (CPT-11)

[0031] 図１～図６に示す通り、ＦＴＤ・ＴＰＩ配合剤が７５～１５０ｍｇ／ｋｇ／ｄａｙ、ＣＰＴ－１１が２５～５０ｍｇ／ｋｇ／ｄａｙのとき、相乗的な抗腫瘍効果が得られた。

[0032] 実施例２

ヒト大腸癌株（ＫＭ２０Ｃ）の培養細胞（１×１０^{7}ｃｅｌｌｓ／マウス）を生後５～６週齢のＢＡＬＢ／ｃＡ　Ｊｃｌ－ｎｕマウスの腹腔内に移植し、各群の平均体重が均等になるように各群にマウスを割り付け、群分け（ｎ＝１０）を実施した日をＤａｙ０とした。

ＦＴＤ・ＴＰＩ配合剤（ＦＴＤとＴＰＩのモル比１：０．５の混合物）は、ＦＴＤとして１５０ｍｇ／ｋｇ／ｄａｙ（推奨投与量）となるように調製した。ＣＰＴ－１１（カンプト注（登録商標）、株式会社ヤクルト本社）は、イリノテカン塩酸塩水和物として５０、７０及び１００ｍｇ／ｋｇ／ｄａｙとなるように調製した。各併用投与群では、Ｄａｙ３から併用投与を開始し、ＦＴＤ・ＴＰＩ配合剤は５日間連日経口投与・２日間休薬を６週間行い、ＣＰＴ－１１は週に１回の尾静脈から投与を６週間行った。

抗腫瘍効果の指標として、Ｄａｙ７０での各群のマウスの生存数を確認し、各群の生存率を比較した。Ｄａｙ７０での各群の生存率を図７に示す。

[0033] 図７の通り、ＦＴＤ・ＴＰＩ配合剤が１５０ｍｇ／ｋｇ／ｄａｙ、ＣＰＴ－１１が５０又は７０ｍｇ／ｋｇ／ｄａｙの群では、Ｄａｙ７０での生存率が１００％であったのに対し、ＦＴＤ・ＴＰＩ配合剤が１５０ｍｇ／ｋｇ／ｄａｙ、ＣＰＴ－１１が１００ｍｇ／ｋｇ／ｄａｙの群では、副作用が強く現れ、Ｄａｙ７０での生存率が３０％まで極端に低下した。

[0034] 実施例３

実施例１に準じて、細胞株をヒト胃癌株（ＳＣ－２）に代えてＦＴＤ・ＴＰＩ配合剤とＣＰＴ－１１の併用投与試験を行った。ＦＴＤ・ＴＰＩ配合剤（ＦＴＤとＴＰＩのモル比１：０．５の混合物）は、ＦＴＤとして７５及び１５０ｍｇ／ｋｇ／ｄａｙ（推奨投与量）、ＣＰＴ－１１は、イリノテカン塩酸塩水和物として４０及び８０ｍｇ／ｋｇ／ｄａｙとなるように調製した。結果を表３に示す。

[0035] [表3]

Drug	Dose (mg/kg/day)	Treatment	RTV [a] (mean ± SD)	TGI [b] (%)
Control	–	–	12.59 ± 0.87	-
FTD·TPI	75	Day1~14, p.o., b.i.d.	6.99 ± 0.45 **	44.5
FTD·TPI	150		4.91 ± 0.28 **	61.0
CPT-11	40	Day1,8, i.v., q.d.	4.46 ± 0.47 **	64.6
CPT-11	80		2.59 ± 0.21 **	79.4
FTD·TPI+CPT-11	75+40	Day1~14, p.o., b.i.d.(FTD·TPI)	2.39 ± 0.35 **##	81.0
FTD·TPI+CPT-11	75+80	Day1,8, i.v., q.d.(CPT-11)	1.24 ± 0.19 **##	90.2
FTD·TPI+CPT-11	150+40	Day1~14, p.o., b.i.d.(FTD·TPI)	1.52 ± 0.15 **##	87.9
FTD·TPI+CPT-11	150+80	Day1,8, i.v., q.d.(CPT-11)	0.95 ± 0.10 **##	92.5

**: $p<0.01$ with Aspin-Welch's t-test as compared with the control group.

##: overall maximal $p<0.01$ by closed testing procedure (Intersection-Union Test).

a) : Relative tumor volume (RTV) on Day 15 was calculated as the ratio of TV on Day 15 to that on Day 0 according to th
RTV = (TV on Day 15) / (TV on Day 0)

b) : Tumor growth inhibition rate (TGI) on Day 15 on the basis of RTV was calculated according to the following formula
TGI (%) = [1 - (mean RTV of the treated group) / (mean RTV of the control group)] × 100

表３のとおり、胃癌においても、単独療法時の推奨投与量の５０～１００％のＦＴＤ・ＴＰＩ配合剤と、単独療法時の推奨投与量の４０～８０％のＣ

ＰＴ－１１を併用した場合、顕著な抗腫瘍効果の増強が確認された。また、体重減少についても許容できる範囲内であった。

[0036] 以上から、単独療法時の推奨投与量の５０～１００％のＦＴＤ・ＴＰＩ配合剤と、単独療法時の推奨投与量の２５～８０％のＣＰＴ－１１を併用した場合、副作用の発症を抑えつつ、優れた抗腫瘍効果を発揮することが明らかになった。

請求の範囲

[請求項1] トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／m^2／ｄａｙ投与され、イリノテカン塩酸塩水和物が４５～１４４ｍｇ／m^2／ｄａｙ投与されることを特徴とする固形癌に対する抗腫瘍剤。

[請求項2] トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤が、トリフルリジン換算量で７０ｍｇ／m^2／ｄａｙで投与される請求項１記載の抗腫瘍剤。

[請求項3] イリノテカン塩酸塩水和物が、７５～１２０ｍｇ／m^2／ｄａｙ投与される請求項１又は２記載の抗腫瘍剤。

[請求項4] 固形癌が、結腸直腸癌、肺癌、乳癌、膵癌又は胃癌である請求項１～３のいずれかに記載の抗腫瘍剤。

[請求項5] α，α，α－トリフルオロチミジン及び５－クロロ－６－（２－イミノピロリジン－１－イル）メチル－２，４（１Ｈ，３Ｈ）－ピリミジンジオン塩酸塩をモル比１：０．５で含有する配合剤を第１日目から第５日目までと第８日目から第１２日目まで投与し、ＣＰＴ－１１を第１日目と第１５日目に投与する２８日間１サイクルの投与スケジュールを１回又は２回以上繰り返すことを特徴とする請求項１～４のいずれかに記載の抗腫瘍剤。

[請求項6] 固形癌患者に対するイリノテカン塩酸塩水和物の抗腫瘍効果を増強するための、トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤からなる抗腫瘍効果増強剤であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／m^2／ｄａｙ投与され、イリノテカン塩酸塩水和物が４５～１４４ｍｇ／m^2／ｄａｙ投与されることを特徴とする抗腫瘍効果増強剤。

[請求項7] イリノテカン塩酸塩水和物を投与された固形癌患者を治療するための、トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有

する配合剤からなる抗腫瘍剤であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／m^2／ｄａｙ投与され、

イリノテカン塩酸塩水和物が４５～１４４ｍｇ／m^2／ｄａｙ投与されることを特徴とする抗腫瘍剤。

[請求項8] トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤を含む抗腫瘍剤と使用説明書を含むキット製剤であって、

使用説明書には、固形癌患者に対して、トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／m^2／ｄａｙ投与され、

イリノテカン塩酸塩水和物が４５～１４４ｍｇ／m^2／ｄａｙ投与されることが記載されていることを特徴とするキット製剤。

[請求項9] 固形癌治療のためのトリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／m^2／ｄａｙ投与され、イリノテカン塩酸塩水和物が４５～１４４ｍｇ／m^2／ｄａｙ投与されることを特徴とする配合剤。

[請求項10] トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤が、トリフルリジン換算量で７０ｍｇ／m^2／ｄａｙで投与される請求項９記載の配合剤。

[請求項11] イリノテカン塩酸塩水和物が、７５～１２０ｍｇ／m^2／ｄａｙ投与される請求項９又は１０記載の配合剤。

[請求項12] 固形癌が、結腸直腸癌、肺癌、乳癌、膵癌又は胃癌である請求項９～１１のいずれかに記載の配合剤。

[請求項13] トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤を第１日目から第５日目までと第８日目から第１２日目まで

投与し、ＣＰＴ－１１を第１日目と第１５日目に投与する２８日間１サイクルの投与スケジュールを１回又は２回以上繰り返すことを特徴とする請求項９～１２のいずれかに記載の配合剤。

[請求項14] 固形癌患者に対するイリノテカン塩酸塩水和物の抗腫瘍効果を増強するための、トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／ｍ^2／ｄａｙ投与され、イリノテカン塩酸塩水和物が４５～１４４ｍｇ／ｍ^2／ｄａｙ投与されることを特徴とする配合剤。

[請求項15] イリノテカン塩酸塩水和物を投与された固形癌患者を治療するための、トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／ｍ^2／ｄａｙ投与され、

イリノテカン塩酸塩水和物が４５～１４４ｍｇ／ｍ^2／ｄａｙ投与されることを特徴とする配合剤。

[請求項16] トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する合剤の固形癌に対する抗腫瘍剤製造のための使用であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／ｍ^2／ｄａｙ投与され、イリノテカン塩酸塩水和物が４５～１４４ｍｇ／ｍ^2／ｄａｙ投与されることを特徴とする使用。

[請求項17] トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤が、トリフルリジン換算量で７０ｍｇ／ｍ^2／ｄａｙで投与される請求項１６記載の使用。

[請求項18] イリノテカン塩酸塩水和物が、７５～１２０ｍｇ／ｍ^2／ｄａｙ投

与される請求項１６又は１７記載の使用。

[請求項19] 固形癌が、結腸直腸癌、肺癌、乳癌、膵癌又は胃癌である請求項１６～１８のいずれかに記載の使用。

[請求項20] トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤を第１日目から第５日目までと第８日目から第１２日目まで投与し、ＣＰＴ－１１を第１日目と第１５日目に投与する２８日間１サイクルの投与スケジュールを１回又は２回以上繰り返すことを特徴とする請求項１６～１９のいずれかに記載の使用。

[請求項21] 固形癌患者に対するイリノテカン塩酸塩水和物の抗腫瘍効果を増強するための、トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤からなる抗腫瘍効果増強剤製造のための使用であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／m^2／ｄａｙ投与され、イリノテカン塩酸塩水和物が４５～１４４ｍｇ／m^2／ｄａｙ投与されることを特徴とする使用。

[請求項22] イリノテカン塩酸塩水和物を投与された固形癌患者を治療するための、トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤からなる抗腫瘍剤製造のための使用であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／m^2／ｄａｙ投与され、

イリノテカン塩酸塩水和物が４５～１４４ｍｇ／m^2／ｄａｙ投与されることを特徴とする使用。

[請求項23] 固形癌患者に対して、トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／m^2／ｄａｙ投与され、イリノテカン塩酸塩水和物が４５～１４４ｍｇ／m^2／ｄａｙ投与されることを特徴とする固形癌の治療方

法。

[請求項24] トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤が、トリフルリジン換算量で７０ｍｇ／ｍ^2／ｄａｙで投与される請求項２３記載の方法。

[請求項25] イリノテカン塩酸塩水和物が、７５～１２０ｍｇ／ｍ^2／ｄａｙ投与される請求項２３又は２４記載の方法。

[請求項26] 固形癌が、結腸直腸癌、肺癌、乳癌、膵癌又は胃癌である請求項２３～２５のいずれかに記載の方法。

[請求項27] トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤を第１日目から第５日目までと第８日目から第１２日目まで投与し、ＣＰＴ－１１を第１日目と第１５日目に投与する２８日間１サイクルの投与スケジュールを１回又は２回以上繰り返すことを特徴とする請求項２３～２６のいずれかに記載の方法。

[請求項28] 固形癌患者に対するイリノテカン塩酸塩水和物の抗腫瘍効果を増強するための方法であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／ｍ^2／ｄａｙ投与され、イリノテカン塩酸塩水和物が４５～１４４ｍｇ／ｍ^2／ｄａｙ投与されることを特徴とする方法。

[請求項29] イリノテカン塩酸塩水和物を投与された固形癌患者を治療するための方法であって、

トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤がトリフルリジン換算量で３５～７０ｍｇ／ｍ^2／ｄａｙ投与され、

イリノテカン塩酸塩水和物が４５～１４４ｍｇ／ｍ^2／ｄａｙ投与されることを特徴とする方法。

[請求項30] トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤とイリノテカン塩酸塩水和物を併用投与することを特徴とす

る固形癌に対する抗腫瘍剤であって、

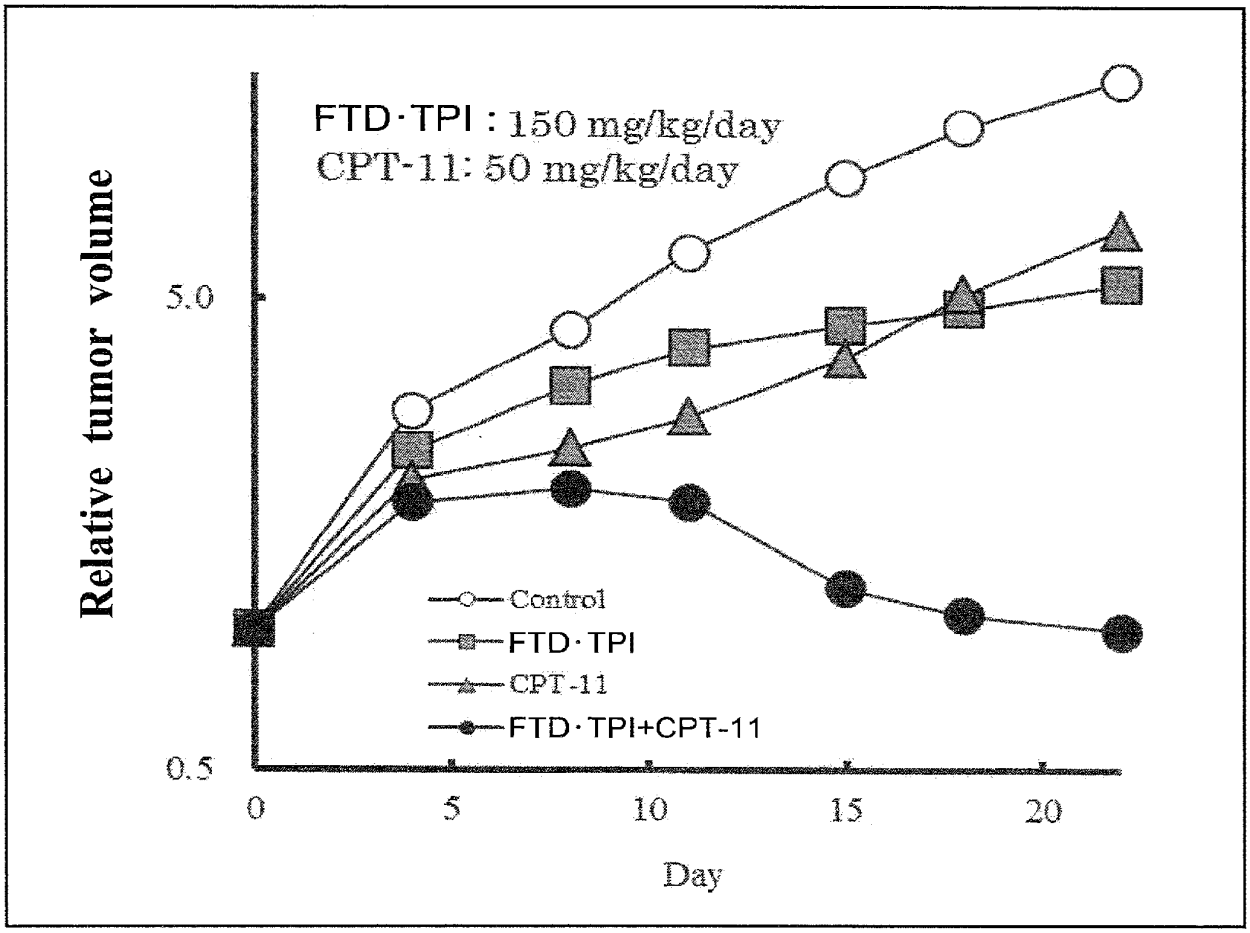
トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤の投与量が、単独療法における推奨投与量の５０％～１００％であり、

イリノテカン塩酸塩水和物の投与量が、単独療法における推奨投与量の２５％～８０％である抗腫瘍剤。

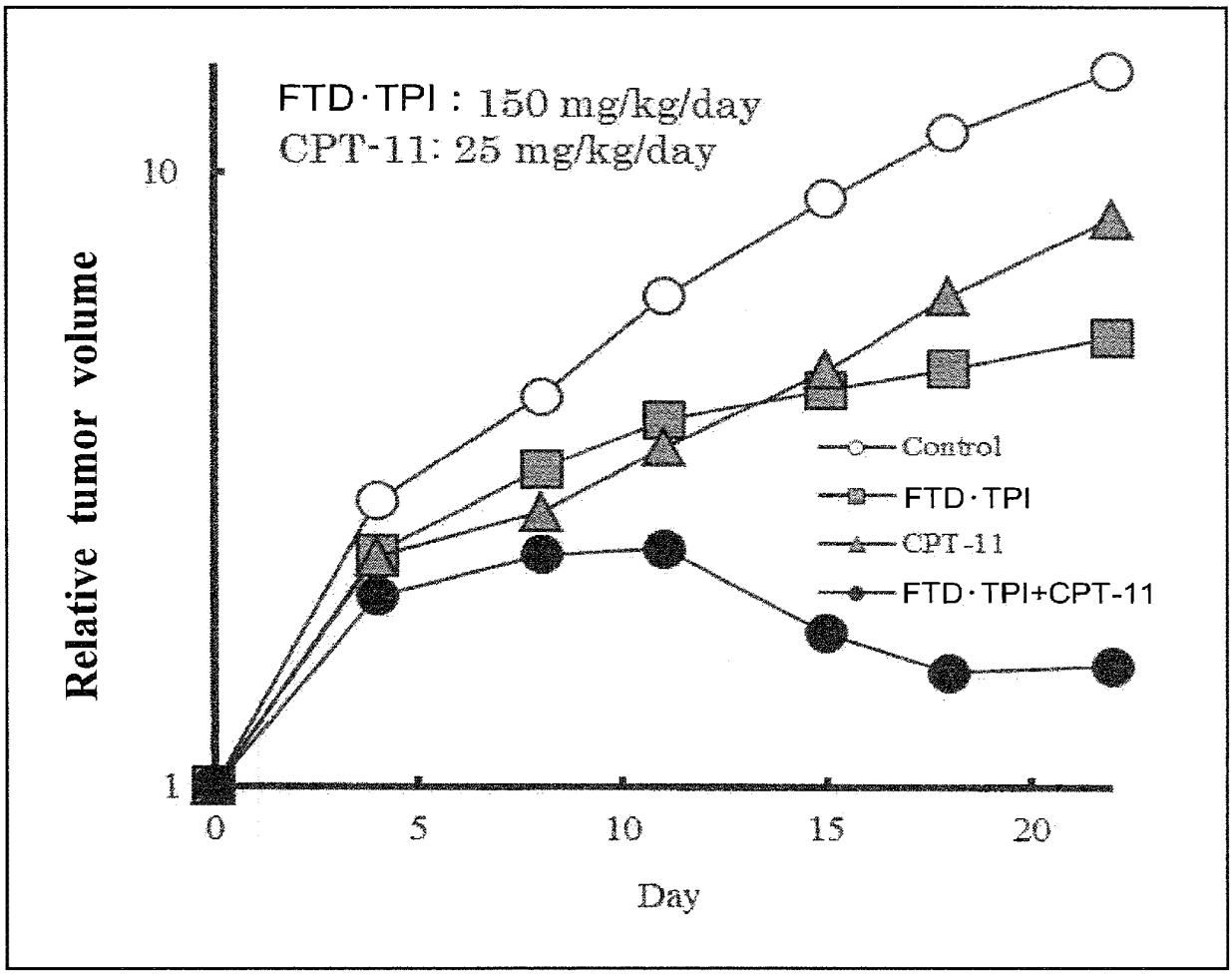
[請求項31] トリフルリジン及びチピラシル塩酸塩をモル比１：０．５で含有する配合剤の投与量が、単独療法における推奨投与量の１００％である請求項３０記載の抗腫瘍剤。

[請求項32] イリノテカン塩酸塩水和物の投与量が、単独療法における推奨投与量の５０％～７０％である請求項３０又は３１記載の抗腫瘍剤。

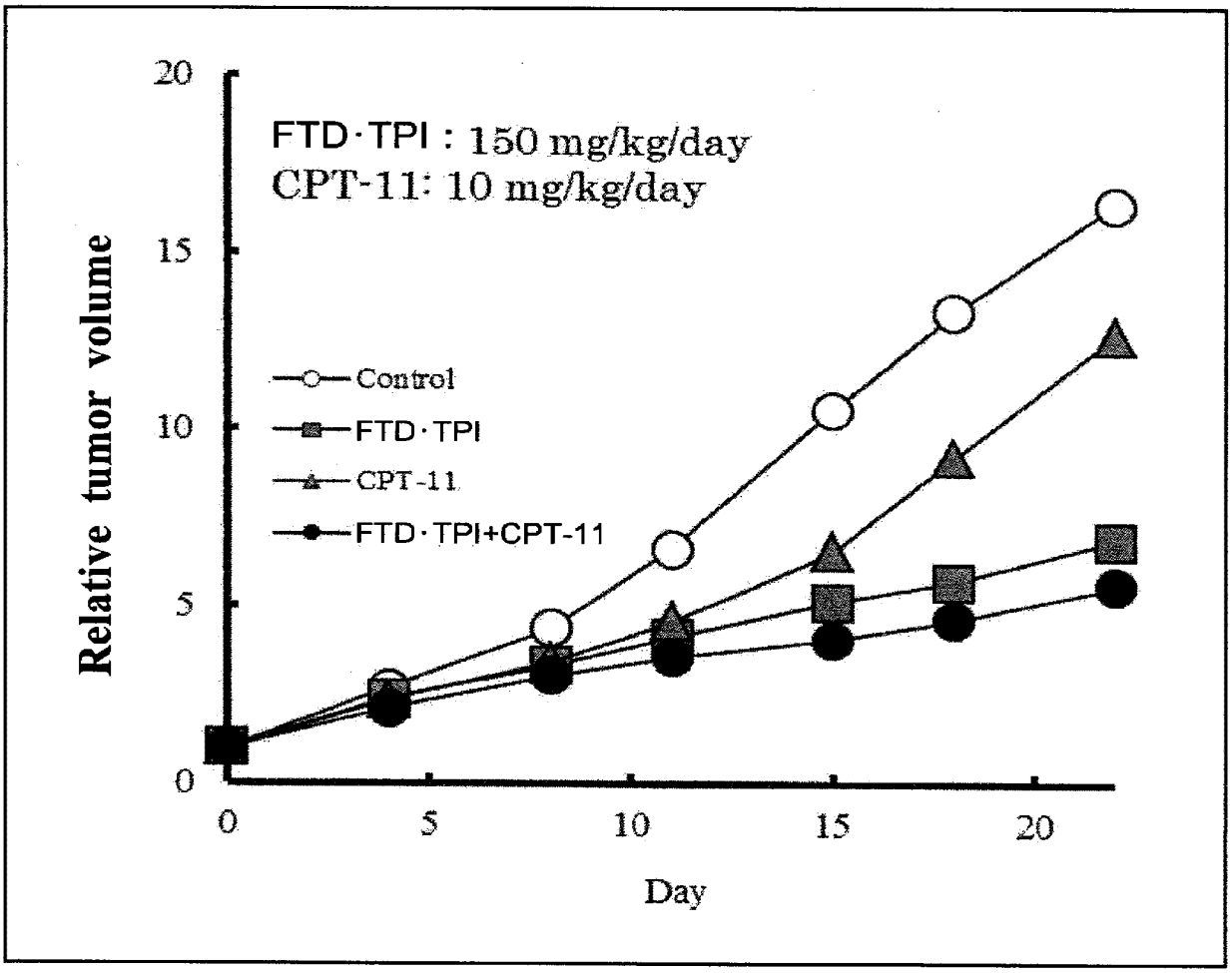
[図1]


FTD·TPI : 150 mg/kg/day
CPT-11: 50 mg/kg/day
Relative tumor volume
5.0
0.5
0
5
10
15
20
Day
Control
FTD·TPI
CPT-11
FTD·TPI+CPT-11

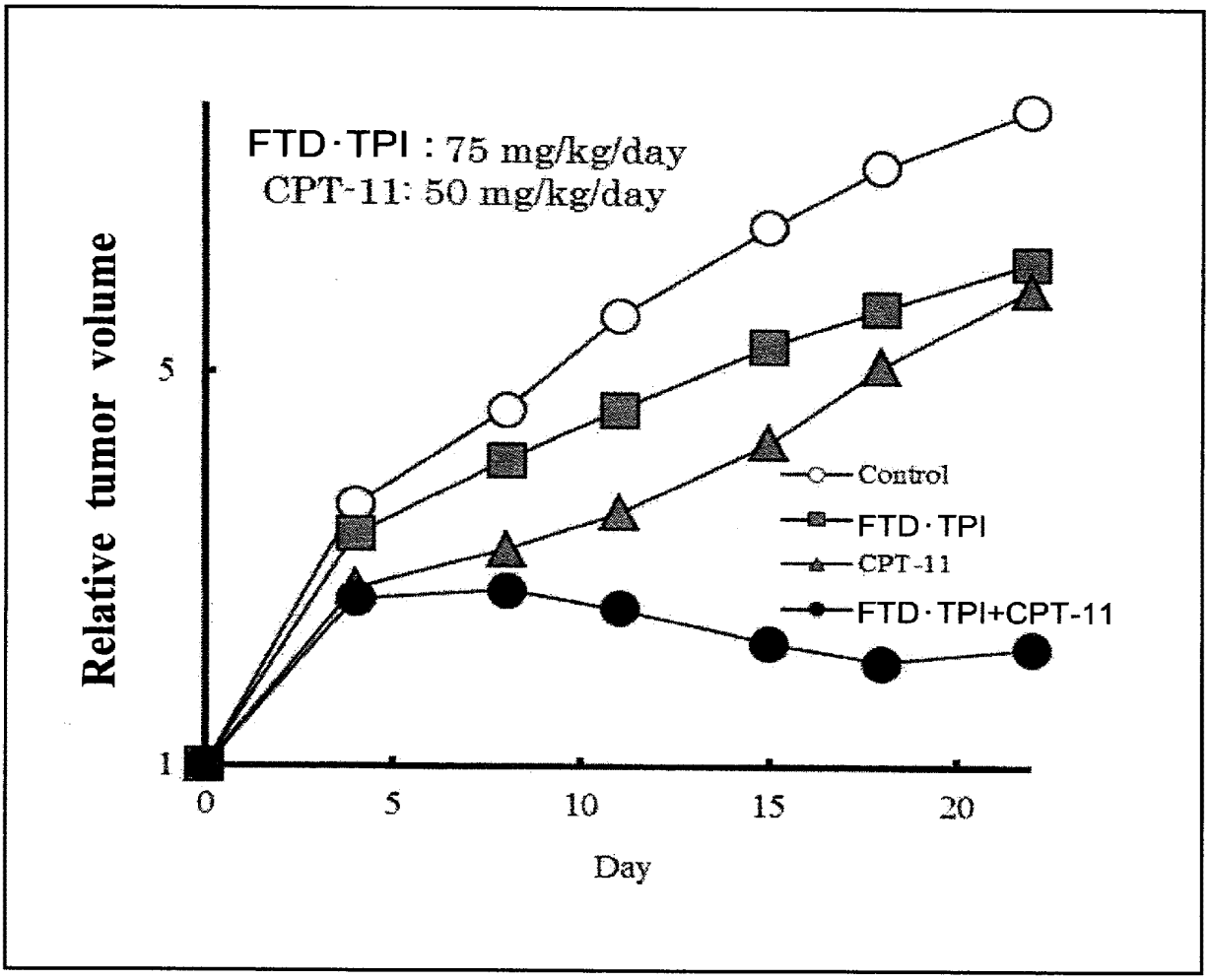
[図2]


FTD·TPI : 150 mg/kg/day
CPT-11: 25 mg/kg/day
Relative tumor volume
10
1
0
5
10
15
20
Day
Control
FTD·TPI
CPT-11
FTD·TPI+CPT-11

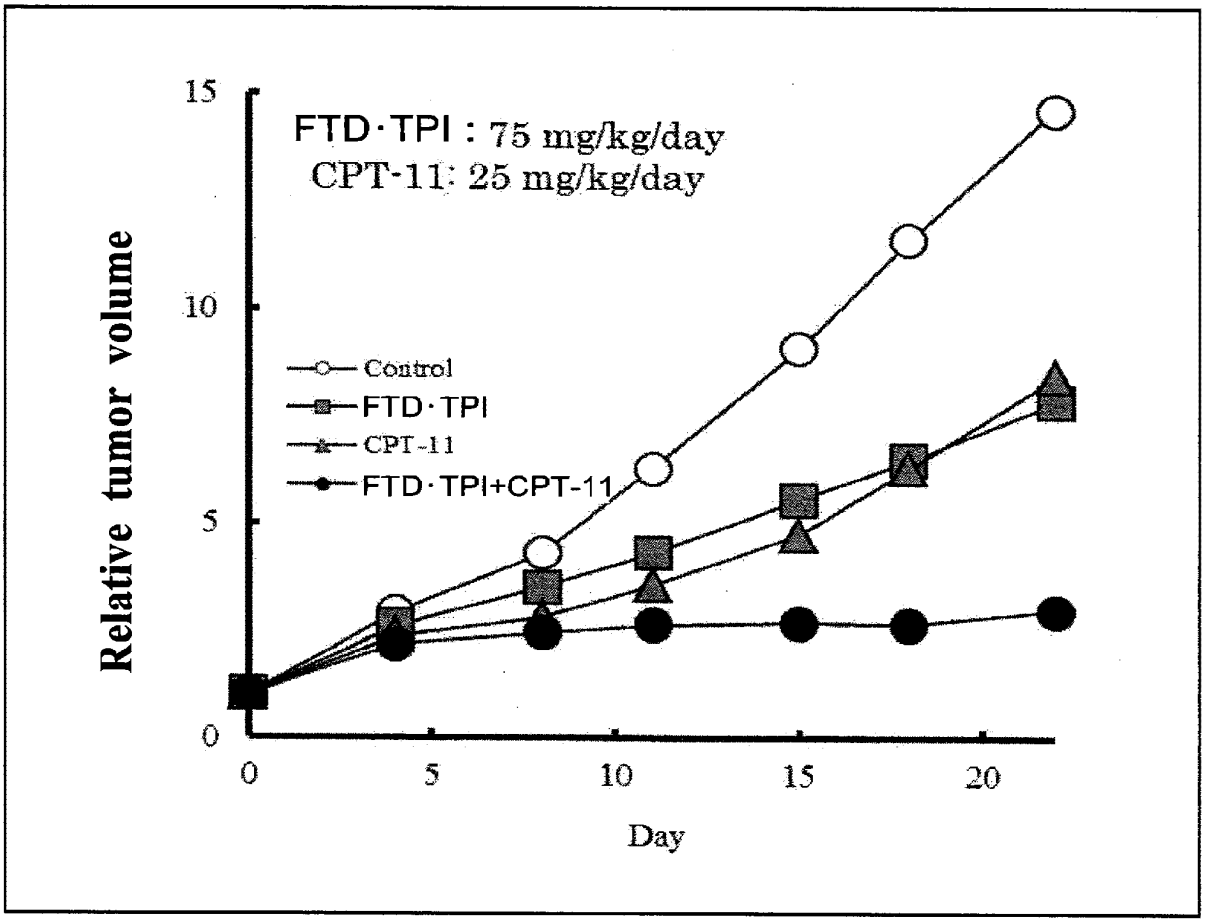
[図3]


FTD·TPI : 150 mg/kg/day
CPT-11: 10 mg/kg/day
Control
FTD·TPI
CPT-11
FTD·TPI+CPT-11
Relative tumor volume
Day
0
5
10
15
20

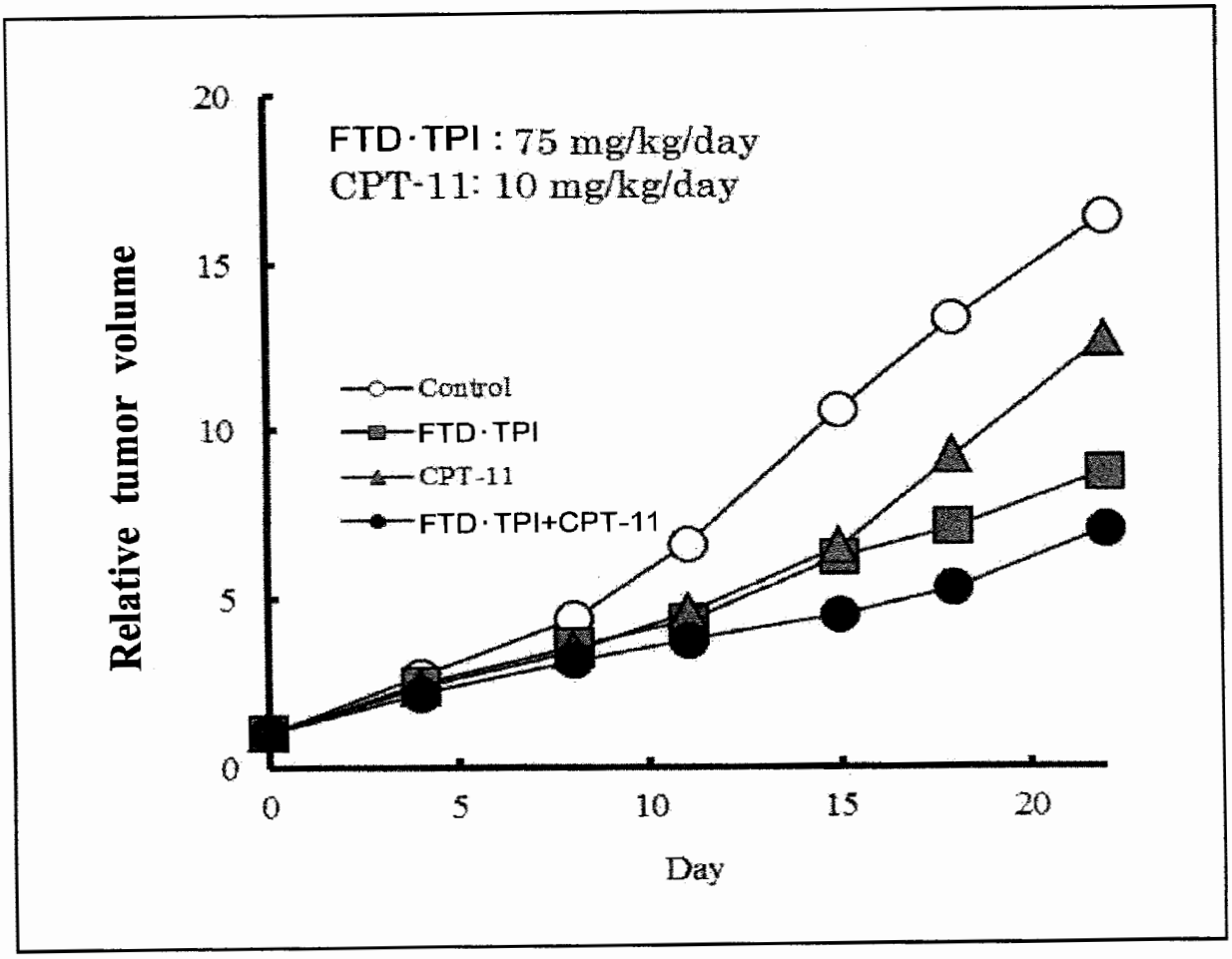
[図4]


FTD·TPI : 75 mg/kg/day
CPT-11: 50 mg/kg/day
Relative tumor volume
5
1
0
5
10
15
20
Day
Control
FTD·TPI
CPT-11
FTD·TPI+CPT-11

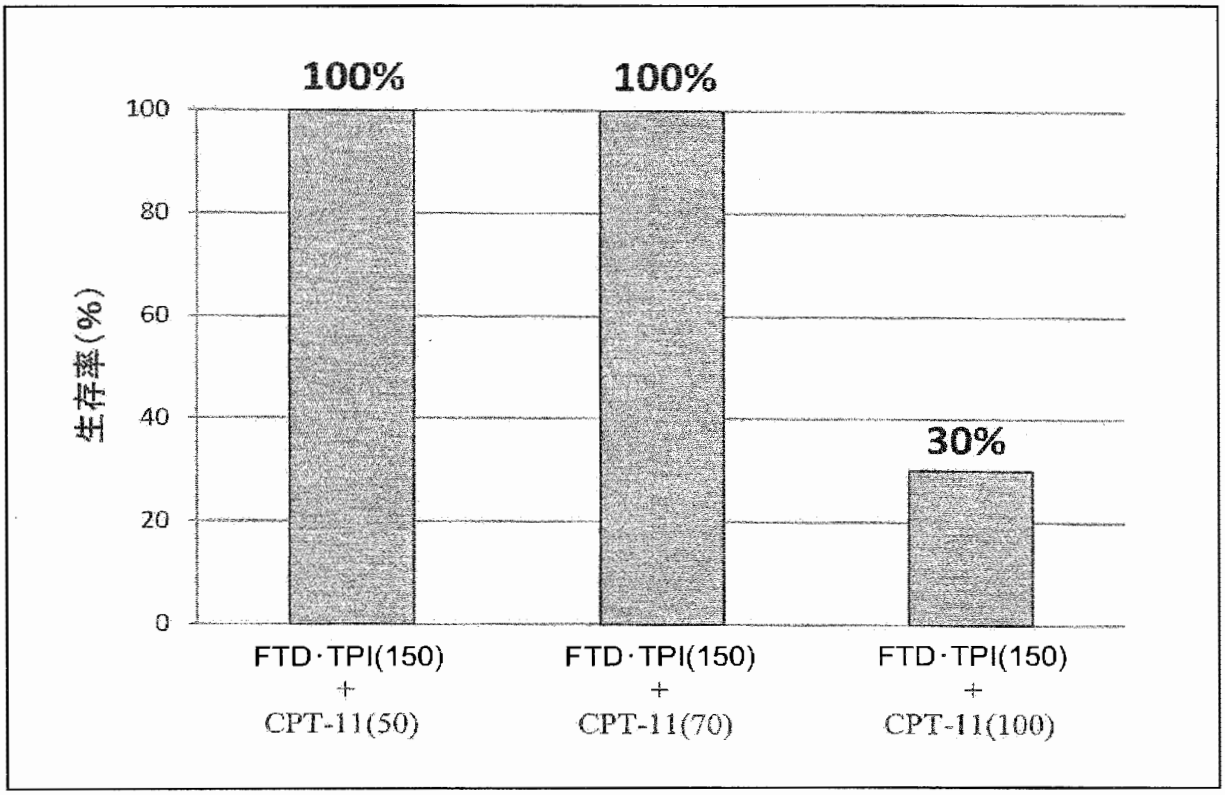
[図5]


FTD·TPI : 75 mg/kg/day
CPT-11: 25 mg/kg/day
Control
FTD·TPI
CPT-11
FTD·TPI+CPT-11
Relative tumor volume
Day
0
5
10
15
20

[図6]


FTD·TPI : 75 mg/kg/day
CPT-11: 10 mg/kg/day
Control
FTD·TPI
CPT-11
FTD·TPI+CPT-11
Relative tumor volume
Day
0
5
10
15
20

[図7]


100%
100%
30%
生存率(%)
100
80
60
40
20
0
FTD·TPI(150) + CPT-11(50)
FTD·TPI(150) + CPT-11(70)
FTD·TPI(150) + CPT-11(100)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2014/058733

A. CLASSIFICATION OF SUBJECT MATTER
A61K31/7072(2006.01)i, *A61K31/4745*(2006.01)i, *A61K31/506*(2006.01)i, *A61P35/00*(2006.01)i, *A61P43/00*(2006.01)i

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)
A61K31/7072, A61K31/4745, A61K31/506, A61P35/00, A61P43/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Jitsuyo Shinan Koho 1922-1996 Jitsuyo Shinan Toroku Koho 1996-2014
Kokai Jitsuyo Shinan Koho 1971-2014 Toroku Jitsuyo Shinan Koho 1994-2014

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAplus/MEDLINE/EMBASE/BIOSIS(STN), JSTPlus/JMEDPlus/JST7580(JDreamIII), Science Direct, WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TEMMINK, O.H.et al, Irinotecan-induced cytotoxicity to colon cancer cells in vitro is stimulated by pre-incubation with trifluorothymidine., European Journal of Cancer, 2007, 43(1), p.175-183, particularly, ABSTRACT	1-22,30-32
Y	WO 2006/080327 A1 (Taiho Pharmaceutical Co., Ltd.), 03 August 2006 (03.08.2006), particularly, scope of claims, claim 1; paragraph [0005] (Family: none)	1-22,30-32

☒ 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
"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 June, 2014 (06.06.14)	17 June, 2014 (17.06.14)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2014/058733

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Ayumu GOTO, "Daicho Gan Kagaku Ryoho -Saikin no Heiyo Toyo- TS-1/Irinotecan Heiyo Ryoho -Genzai no Chiken-", Japanese Journal of Cancer and Chemotherapy, 2006, 33(7), pages 896 to 900, particularly, page 897, right column	1-22,30-32
Y	Sotaro SADAHIRO et al., "Two Patients with Recurrent Colon Cancer Who Underwent Surgery Following a Combination of Irinotecan and UFT", Japanese Journal of Cancer and Chemotherapy, 2002, 29(11), pages 2013 to 2018, particularly, page 2014, left column, 2nd paragraph, right column, 3rd paragraph	1-22,30-32
Y	JP 2011-157298 A (ASKA Pharmaceutical Co., Ltd.), 18 August 2011 (18.08.2011), particularly, paragraph [0007] (Family: none)	30-32
Y	JP 2010-500964 A (DSM IP Assets B.V.), 14 January 2010 (14.01.2010), particularly, paragraph [0016] & US 2010/0056463 A1 & EP 2040696 A & WO 2008/006581 A2 & KR 10-2009-0028836 A & CN 101516364 A	30-32
Y	JP 2009-528340 A (Boulikas, Parthenios), 06 August 2009 (06.08.2009), particularly, paragraph [0069] & US 2009/0053302 A1 & EP 2001441 A & WO 2007/099377 A2 & GR 20060100144 A & NO 20083927 A & CA 2644566 A & KR 10-2009-0023548 A & EA 200801912 A & CN 101522172 A & ZA 200807934 A & RS 20080388 A	30-32
P,X	YAMAZAKI, K.et al, A first combination phase I study of TAS-102 and irinotecan (Iri) in Japanese patients (pts) with metastatic colorectal cancer (mCRC) refractory to fluoropyrimidine (FU) and oxaliplatin (Ox)., European Journal of Cancer, September 2013, 49, Supp. SUPPL. 2, p.S555	1-22,30-32
A	TEMMINK, O.H.et al, Therapeutic potential of the dual-targeted TAS-102 formulation in the treatment of gastrointestinal malignancies., Cancer Sci., 2007, 98(6), p.779-789	1-22,30-32

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2014/058733

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

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

1. [x] Claims Nos.: 23-29
because they relate to subject matter not required to be searched by this Authority, namely:
(See extra sheet)

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

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

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

Remark on Protest

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

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

[ ] No protest accompanied the payment of additional search fees.

Continuation of Box No.II-1 of continuation of first sheet(2)

The inventions of the above-said claims pertain to methods for treatment of the human body or animal body by surgery or therapy and thus relate to a subject matter on which this International Searching Authority is not required to carry out an international search under the provisions of PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv).

国際調査報告

国際出願番号　ＰＣＴ／ＪＰ２０１４／０５８７３３

Ａ．　発明の属する分野の分類（国際特許分類（ＩＰＣ））

Int.Cl.　A61K31/7072(2006.01)i, A61K31/4745(2006.01)i, A61K31/506(2006.01)i, A61P35/00(2006.01)i, A61P43/00(2006.01)i

Ｂ．　調査を行った分野

調査を行った最小限資料（国際特許分類（ＩＰＣ））

Int.Cl.　A61K31/7072, A61K31/4745, A61K31/506, A61P35/00, A61P43/00

最小限資料以外の資料で調査を行った分野に含まれるもの

日本国実用新案公報	１９２２－１９９６年
日本国公開実用新案公報	１９７１－２０１４年
日本国実用新案登録公報	１９９６－２０１４年
日本国登録実用新案公報	１９９４－２０１４年

国際調査で使用した電子データベース（データベースの名称、調査に使用した用語）

ＣＡｐｌｕｓ／ＭＥＤＬＩＮＥ／ＥＭＢＡＳＥ／ＢＩＯＳＩＳ（ＳＴＮ），ＪＳＴＰｌｕｓ／ＪＭＥＤＰｌｕｓ／ＪＳＴ７５８０（ＪＤｒｅａｍＩＩＩ），Ｓｃｉｅｎｃｅ　Ｄｉｒｅｃｔ，ＷＰＩ

Ｃ．　関連すると認められる文献

引用文献のカテゴリー＊	引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示	関連する請求項の番号
Y	TEMMINK, O. H. et al, Irinotecan-induced cytotoxicity to colon cancer cells in vitro is stimulated by pre-incubation with trifluorothymidine., European Journal of Cancer , 2007, 43(1), p. 175-183, 特に ABSTRACT	1-22, 30-32
Y	WO 2006/080327 A1 (大鵬薬品工業株式会社) 2006.08.03　特に特許請求の範囲請求項１、段落[0005] (ファミリーなし)	1-22, 30-32

☑　Ｃ欄の続きにも文献が列挙されている。　　☐　パテントファミリーに関する別紙を参照。

＊　引用文献のカテゴリー
「Ａ」特に関連のある文献ではなく、一般的技術水準を示すもの
「Ｅ」国際出願日前の出願または特許であるが、国際出願日以後に公表されたもの
「Ｌ」優先権主張に疑義を提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献（理由を付す）
「Ｏ」口頭による開示、使用、展示等に言及する文献
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国際調査を完了した日 ０６．０６．２０１４	国際調査報告の発送日 １７．０６．２０１４
国際調査機関の名称及びあて先 日本国特許庁（ＩＳＡ／ＪＰ） 郵便番号１００－８９１５ 東京都千代田区霞が関三丁目４番３号	特許庁審査官（権限のある職員）　４Ｕ　９７３５ 六笠　紀子 電話番号　０３－３５８１－１１０１　内線　３４３９

Ｃ（続き）．　関連すると認められる文献

引用文献のカテゴリー＊	引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示	関連する請求項の番号
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第Ⅱ欄　請求の範囲の一部の調査ができないときの意見（第１ページの２の続き）

法第8条第3項（ＰＣＴ17条(2)(a)）の規定により、この国際調査報告は次の理由により請求の範囲の一部について作成しなかった。

１．☑ 請求項　23-29　は、この国際調査機関が調査をすることを要しない対象に係るものである。
つまり、
上記請求項に係る発明は、手術又は治療による人体又は動物の体の処置方法に関するものであって、PCT17条(2)(a)(i)及びPCT規則39.1(iv)の規定により、この国際調査機関が国際調査をすることを要しない対象に係るものである。

２．☐ 請求項　　は、有意義な国際調査をすることができる程度まで所定の要件を満たしていない国際出願の部分に係るものである。つまり、

３．☐ 請求項　　は、従属請求の範囲であってＰＣＴ規則6.4(a)の第２文及び第3文の規定に従って記載されていない。

第Ⅲ欄　発明の単一性が欠如しているときの意見（第１ページの３の続き）

次に述べるようにこの国際出願に二以上の発明があるとこの国際調査機関は認めた。

１．☐ 出願人が必要な追加調査手数料をすべて期間内に納付したので、この国際調査報告は、すべての調査可能な請求項について作成した。

２．☐ 追加調査手数料を要求するまでもなく、すべての調査可能な請求項について調査することができたので、追加調査手数料の納付を求めなかった。

３．☐ 出願人が必要な追加調査手数料を一部のみしか期間内に納付しなかったので、この国際調査報告は、手数料の納付のあった次の請求項のみについて作成した。

４．☐ 出願人が必要な追加調査手数料を期間内に納付しなかったので、この国際調査報告は、請求の範囲の最初に記載されている発明に係る次の請求項について作成した。

追加調査手数料の異議の申立てに関する注意

☐ 追加調査手数料及び、該当する場合には、異議申立手数料の納付と共に、出願人から異議申立てがあった。

☐ 追加調査手数料の納付と共に出願人から異議申立てがあったが、異議申立手数料が納付命令書に示した期間内に支払われなかった。

☐ 追加調査手数料の納付はあったが、異議申立てはなかった。

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(54) Title: COMPOSITIONS FOR IMPROVING THE PHARMACOKINETICS AND THERAPEUTIC INDEX OF CANCER TREATMENT

(57) Abstract: Provided are methods for improving the pharmacokinetics of drug therapy, in one aspect methods are provided for co-administration of a liposomally encapsulated, PEGylated, or protein-conjugated drug via a parenteral route and the same drug in free (non-encapsulated PEGylated, or protein-conjugated) form via an enteral or parenteral route, in another aspect, methods are provided for treating a cancer in a patient by co-administering liposomal irinotecan and free irinotecan, optionally in further combination with additional therapeutic agents.

COMPOSITIONS FOR IMPROVING THE PHARMACOKINETICS AND THERAPEUTIC INDEX OF CANCER TREATMENT

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/147,389, filed April 14, 2015, the disclosure of which is hereby incorporated by reference in its entirety.

BACKGROUND

[0002] Colorectal cancer accounts for 10 to 15 percent of all cancers and is the second leading cause of cancer deaths in Western countries. Approximately half of the patients develop metastatic disease. The prognosis for these patients is poor, even though palliative chemotherapy has been able to prolong survival and improve the quality of life over best supportive care. Accordingly, there remains a critical need to further improve therapies so as to prolong patients' lives while maintaining quality of life, particularly in the case of colorectal cancers that often are, or become, resistant to current therapeutic modalities.

SUMMARY

[0003] Provided are methods of improving the pharmacokinetic profile of a drug treatment in a patient (*e.g.*, a human patient), the methods comprising administering to the patient a therapeutically effective amount of the drug, wherein the therapeutically effective amount is administered as a first amount of the drug administered to the patient parenterally in a sustained release dosage form and a second amount of the same drug co-administering to the patient enterally or parenterally in an immediate release dosage form. Beneficially, the first amount combined with the second amount equals a therapeutically effective amount of the drug. The drug may be administered in doses (each dose comprising the first amount and the second amount) separated by an interval of a day or more, or a week, two weeks, or three weeks, or a month or more. In some embodiments, the first amount of the drug is administered parenterally in a form (e.g., a liposomally encapsulated form) that provides sustained release, and the same drug is co-administered parenterally in the second amount, e.g., at the same interval, such that the pharmacokinetic profile is improved. Beneficially, the drug reaches therapeutic levels at a site of action faster than if administered only in the sustained release (e.g., liposomally encapsulated) form, and maintains therapeutic levels at the site of action longer than if administered at the same intervals only in the immediate release form.

[0004] The immediate release form may be a free (non-encapsulated, non-PEGylated, and non-protein-conjugated) form, and the sustained release dosage form may be a liposomally

encapsulated, PEGylated, or protein-conjugated (e.g., albumin-conjugated) form. Such co-administration results in improvement of the pharmacokinetic profile of the drug, or in improvement of the therapeutic index (efficacy to safety ratio) of the drug, or in therapeutic synergy, as compared to administration to matched patients of the first amount and the second amount as a combined amount in the sustained release injectable dosage form.

[0005] The unencapsulated drug and the drug in sustained release dosage form (e.g., liposomally encapsulated, PEGylated, or protein-conjugated form) may be administered sequentially or simultaneously. The sustained release and immediate release dosage forms may both be comprised within a single formulation for simultaneous injection. In one embodiment, the second amount of the immediate release injectable dosage form is at least 10% of the first amount of the sustained release injectable dosage form. The drug may be an anti-cancer drug. In one embodiment, the sustained release dosage form is in a liposomal, hyaluronate, or PEGylated form and the immediate release dosage form is in the form of a hydrochloride or other salt solution, or analogs or derivatives thereof.

[0006] In various embodiments the drug is an anti-cancer drug. The anti-cancer drug may be selected from a topoisomerase inhibitor (e.g., a topoisomerase I inhibitor or a topoisomerase II inhibitor), an anti-microtubule agent (e.g., a taxane, a vinca alkaloid, a vinca alkaloid derivative), an anti-metabolite (e.g., an anti-folate, a fluoropyrimidine, a deoxynucleoside analogue, or a thiopurine), a platinum salt, an alkylating agent (e.g., a nitrogen mustard, a nitrosourea, a tetrazine, an aziridine, an organoplatinum agent, procarbazine or hexamethylmelamine), an anti-angiogenic agent, or a cytotoxic antibiotic. The topoisomerase I inhibitor may be, e.g., camptothecin, irinotecan, SN-38, or topotecan; the topoisomerase II inhibitor may be , e.g., pixantrone, mitoxantrone, or an anthracycline (e.g., doxorubicin, daunorubicin, bleomycin, dactinomycin, epirubicin, or idarubicin) or it may be, e.g., etoposide or teniposide; the taxane may be, e.g., paclitaxel, docetaxel, cabazitaxel, or tesetaxel, the vinca alkaloid is, e.g., vincristine, vinblastine, or vinorelbine, the platinum salt may be, e.g., oxaliplatin, cisplatin or carboplatin, the alkylating agent may be, e.g., bendamustine, busulfan, carmustine, chlormbucil, cyclophosphamide, ifosfamide, lomustine, mechlorethamine, melphalan, streptazocin, thiotepa, or uramustine, the anti-metabolite may be, e.g., 5-FU, capecitabine, cytarabine, gemcitabine, methotrexate, pemetrexed, or tegafur, and the anti-angiogenic agent may be, e.g., bevacizumab. The liposomally encapsulated irinotecan may be irinotecan sucrosofate liposome injection (nal-IRI, ONIVYDE™, MM-398).

[0007] Exemplary combinations include: liposomal irinotecan (e.g., MM-398 or IHL-305) co-administered with SN-38 or free irinotecan (e.g., CAMPTOSAR®); hyaluronate irinotecan (HA-

irinotecan) co-administered with SN-38 or irinotecan; PEGylated irinotecan (e.g., NKTR-102) co-administered with SN-38 or irinotecan; cyclodextrin camptothecin (e.g., CRLX101) co-administered with camptothecin; or PEGylated SN-38 (e.g., NK102 or EZN2208) co-administered with SN-38 or irinotecan. Liposomal doxorubicin (e.g., DOXIL®, CAELYX®, MYOCET®) co-administered with doxorubicin; liposomal daunorubicin (e.g., DAUNOSOME®) co-administered with daunorubicin, bleomycin, dactinomycin, epirubicin, idarubicin, or mitoxantrone. Albumin conjugated paclitaxel (e.g., ABRAXANE®) co-administered with paclitaxel; liposomal docetaxel co-administered with docetaxel; cyclodextrin docetaxel co-administered with docetaxel; PEGylated docetaxel co-administered with docetaxel. Liposomal vincristine (e.g., MARQIBO®) co-administered with vincristine; liposomal vinorelbine co-administered with vinorelbine; liposomal vinblastine co-administered with vinblastine. Liposomal or PEGylated or protein-conjugated oxaliplatin co-administered with oxaliplatin; liposomal or PEGylated or protein-conjugated cisplatin co-administered with cisplatin; liposomal or PEGylated or protein-conjugated carboplatin co-administered with carboplatin.

[0008] Also provided are methods for treating cancers (tumors, e.g., unresectable tumors), in a patient (*e.g.*, a human patient) comprising co-administering to the patient liposomal irinotecan (e.g., ONIVYDE™) and free irinotecan (*i.e.*, in an unencapsulated solution or suspension, e.g., CAMPTOSAR®), optionally in combination with co-administration of 5-fluorouracil (5-FU) and leucovorin, optionally in further combination with co-administration of bevacizumab, according to particular clinical dosage regimens providing effective amounts of each drug. Compositions adapted for use in such methods also are provided.

[0009] In one aspect, a method for treatment (*e.g.*, effective treatment) of a cancer (e.g., an unresectable cancer) in a patient is provided, the method comprising: administering to the patient an effective amount of irinotecan, where the effective amount is comprised by a combination of liposomal irinotecan co-administered with free (non-encapsulated) irinotecan, wherein the method comprises at least one cycle, wherein the cycle is a period of two weeks, and wherein for each cycle the liposomal irinotecan is administered to patients at a dose of 60 mg/m^2 or 80 mg/m^2, and the free irinotecan is administered to patients at a dose of 90 mg/m^2 or 120 mg/m^2.

[0010] In one embodiment, the cancer is a gastrointestinal cancer, *e.g.*, colorectal cancer (CRC). In another embodiment, the cancer is metastatic CRC. In another embodiment, the cancer is pancreatic cancer. In yet another embodiment, the patient previously has been treated for the cancer with first line standard of care therapy.

[0011] In another aspect, a method for treatment of CRC (optionally unresectable advanced CRC) in a patient is provided, the method comprising: co-administrating to a patient an effective amount of each liposomal irinotecan, free irinotecan, 5-fluorouracil (5-FU), leucovorin, and optionally bevacizumab, wherein the method comprises at least one cycle of co-administration, wherein a cycle is a period of two weeks, and wherein for each cycle:

[0012] (a) liposomal irinotecan is administered once to patients at a dose of 60 mg/m^2 or 80 mg/m^2;

[0013] (b) free irinotecan is administered once to patients at a dose of 90 mg/m^2 or 120 mg/m^2;

[0014] (c) leucovorin is administered once to patients at a dose of 400 mg/m^2;

[0015] (d) 5-FU is administered once to patients at a dose of 2400 mg/m^2; and

[0016] (e) bevacizumab is optionally administered once to patients at a dose of 5 mg/kg or 10 mg/kg.

[0017] In one embodiment, the liposomal irinotecan is administered intravenously over 90 minutes. In another embodiment, the 5-FU is administered intravenously over 46 hours. In another embodiment, the leucovorin is administered intravenously over 2 hours. In another embodiment, the bevacizumab is administered intravenously over 30-90 minutes. In another embodiment, the liposomal irinotecan is administered intravenously over 60 minutes or 90 minutes. In another embodiment, the free irinotecan is administered intravenously over 60 minutes.

[0018] In other embodiments, the liposomal irinotecan is administered prior to the free irinotecan. In one embodiment, the leucovorin and 5-FU are administered sequentially. The leucovorin and 5-fluorouracil may be administered after the two formulations of irinotecan, with the leucovorin administered before the 5-FU. In another embodiment, the bevacizumab is administered prior to the liposomal irinotecan, the free irinotecan, the leucovorin, and the 5-FU.

[0019] In still other embodiments, treating the patient results in a positive outcome, wherein the positive outcome is pathologic complete response (pCR), complete response (CR), partial response (PR) or stable disease (SD). In one embodiment, the combination therapy with liposomal irinotecan, free irinotecan, 5-FU and leucovorin results in therapeutic synergy.

[0020] In further embodiments, the liposomal irinotecan is formulated as irinotecan liposome injection (MM-398, ONIVYDE™). MM-398 may also be referred to as irinotecan HCl liposome injection because irinotecan HCl is the active pharmaceutical ingredient that is used to load irinotecan into liposomes containing triethylammonium sucrose octasulfate to prepare MM-398 liposomes. This nomenclature may be used even though the hydrochloride ion of the irinotecan

HCl reacts with the triethylammonium ion of the triethylammonium sucrose octasulfate to yield triethylammonium chloride (triethylamine hydrochloride) which may diffuse out of the liposomes, leaving irinotecan sucrosofate entrapped within the MM-398 liposomes.

[0021] In another aspect, a formulation of liposomal irinotecan (e.g., MM-398) is provided for co-administration with free irinotecan in at least one cycle, wherein the cycle is two weeks, and wherein:

[0022] (a) liposomal irinotecan is administered at a dose of 60 or 80 mg/m^2; and

[0023] (b) free irinotecan is administered at a dose of 90 or 120 mg/m^2.

[0024] In another aspect, a formulation of liposomal irinotecan is provided for co-administration with free irinotecan, 5-fluorouracil (5-FU), leucovorin, and bevacizumab in at least one cycle is provided, wherein the cycle is two weeks, and wherein:

[0025] (a) liposomal irinotecan is administered at a dose of 60 or 80 mg/m^2;

[0026] (b) free irinotecan is administered at a dose of 90 or 120 mg/m^2;

[0027] (c) leucovorin is administered at a dose of 400 mg/m^2;

[0028] (d) 5-fluorouracil is administered at a dose of 2400 mg/m^2; and

[0029] (e) bevacizumab is administered at a dose of 5 mg/kg.

[0030] Optionally, the liposomal irinotecan is administered intravenously over 90 minutes, and/or the 5-FU is administered intravenously over 46 hours, and/or the leucovorin is administered intravenously over 2 hours. In an alternate embodiment, the liposomal irinotecan is administered intravenously over 6 minutes or 90 minutes and/or the free irinotecan is administered intravenously over 60 minutes.

[0031] In another aspect, the camptothecin topoisomerase I inhibitor is irinotecan and the sustained release dosage form is in a liposomal, hyaluronate, or PEGylated form, and the immediate release dosage form is in the form of an irinotecan hydrochloride solution or its analogs or derivatives. In one embodiment, a cycle is a period of two weeks, and for each co-administration:

[0032] (a) the liposomal irinotecan sucrosofate is administered at a dose range between 60 and 100 mg/m^2;

[0033] (b) the irinotecan hydrochloride is administered at a dose range between 90 and 180 mg/m^2;

[0034] (c) the leucovorin is administered at a dose of 400 mg/m^2;

[0035] (d) the 5-fluorouracil is administered at a dose of 2400 mg/m^2; and

[0036] (e) the bevacizumab is co-administered at a dose of 5 mg/kg Q2W or 10 mg/kg Q2w or 15 mg/kg Q3W.

[0037] In another aspect, a kit for treating CRC (e.g., unresectable CRC, metastatic CRC, or metastatic unresectable CRC) in a patient is provided, the kit comprising a first container, and within the first container, a second container and a third container, the second container holding a dose of liposomal irinotecan and the third container holding a dose of free irinotecan. The kit optionally further comprising instructions for using liposomal irinotecan, free irinotecan, and optionally 5-FU and leucovorin, and optionally bevacizumab, as described herein.

[0038] An MM-398 PEGylated liposome encapsulating irinotecan and sucrose octasulphate can be used in a method of treating unresectable, advanced cancer in a human patient, the method comprising administration to the human patient once every two weeks in a treatment cycle.

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] Figures 1A - 1C summarize MM-398 pharmacokinetic parameters in an HT-29 xenograft study.

[0040] Figures 1A and 1B are graphs showing plasma irinotecan and SN-38 levels, respectively, following free irinotecan and MM-398 administrations. Figure 1C is a graph showing intratumor levels of irinotecan and SN-38 levels following different doses of MM-398.

DETAILED DESCRIPTION

[0041] *Definitions*

[0042] The term "patient" is a human patient.

[0043] The term "effective treatment" refers to treatment producing a beneficial effect, e.g., amelioration of at least one symptom of a disease or disorder. A beneficial effect can take the form of an improvement over baseline, i.e., an improvement over a measurement or observation made prior to initiation of therapy according to the method. A beneficial effect can also take the form of arresting, slowing, retarding, or stabilizing of a deleterious progression of a marker of a cancer. Effective treatment may refer to alleviation of at least one symptom of a cancer. Such effective treatment may, e.g., reduce patient pain, reduce the size and/or number of lesions, may reduce or prevent metastasis of a cancer tumor, and/or may slow growth of a cancer tumor. Effective treatment with a drug or composition may: (i) reduce the number of cancer cells; (ii) reduce tumor size; (iii) inhibit, retard, slow to some extent and may stop cancer cell infiltration into peripheral organs; (iv) inhibit (i.e., slow to some extent and may stop) tumor metastasis; (v) inhibit tumor growth; (vi) prevent or delay occurrence and/or recurrence of tumor; and/or (vii) relieve to some extent one or more of the symptoms associated with the cancer.

[0044] The terms "combination therapy," "co-administration," or "co-administered" (or minor variations of these terms) include simultaneous administration of at least two therapeutic agents

to a patient or their sequential administration within a time period during which the first administered therapeutic agent is still present in the patient when the second administered therapeutic agent is administered.

[0045] The term "monotherapy" refers to administering a single drug preparation containing a single drug to treat a disease or disorder in the absence of co-administration of any other drug or therapeutic preparation to treat the same disease or disorder.

[0046] "Dosage" refers to parameters for administering a drug preparation in defined quantities per unit time (*e.g.*, per hour, per day, per week, per month, etc.) to a patient. Such parameters include, *e.g.*, the quantity of drug in each dose or administration. Such parameters also include the configuration of each dose, which may be administered as one or more units, *e.g.*, each taken at a single administration, *e.g.*, injected (*e.g.*, as an infusion or a bolus). Such parameters further include frequency of administration of separate doses, which frequency may change over time.

[0047] "Dosage form" refers to the form and/or formulation in which a drug is provided for use, e.g. a free aqueous solution, an aqueous suspension, a liposomal suspension, a single dose vial, a pill, a capsule, etc.

[0048] "Dose" refers to an amount of a drug given in a single administration.

[0049] The terms "resistant" and "refractory" refer to tumor cells that continue to grow or divide during treatment with a therapeutic agent. Such cells may have responded (*e.g.*, by not growing or dividing) to a therapeutic agent initially, but subsequently exhibited a reduction of responsiveness during treatment.

[0050] "Therapeutic synergy" refers to a phenomenon where treatment of patients with a combination of therapeutic agents (e.g., co-administration of a combination of different drugs or co-administration of a combination of different formulations of the same drug) manifests a therapeutically superior outcome to the outcome achieved by each individual constituent of the combination used at its optimum dose. In this context a therapeutically superior outcome is one in which the patients either a) exhibit fewer incidences of adverse events while receiving a therapeutic benefit that is equal to or greater than that where individual constituents of the combination are each administered as monotherapy at the same dose as in the combination, or b) do not exhibit dose-limiting toxicities while receiving a therapeutic benefit that is greater than that of treatment with each individual constituent of the combination when each constituent is administered in at the same doses in the combination(s) as is administered as individual components. In xenograft models, a combination, used at its maximum tolerated dose, in which each of the constituents will be present at a dose generally not exceeding its individual maximum tolerated dose, manifests therapeutic synergy when decrease in tumor growth achieved by

administration of the combination is greater than the value of the decrease in tumor growth of the best constituent when the constituent is administered alone.

[0051] *II. Improving the pharmacokinetic profile of drug treatment*

[0052] Unencapsulated drugs typically provide rapid exposure to high drug levels, but often do not sustain exposure over a long time period. In a complementary fashion, encapsulation of a drug can be engineered so as to provide a sustained release pharmacokinetic profile, sustaining exposure for a prolonged period of time. Such an encapsulated drug may, however, take longer to reach therapeutic levels at the site of action than the free drug.

[0053] *III. Irinotecan and MM-398*

[0054] Irinotecan is (*S*)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1H-pyrano[3',4':6,7]-indolizino[1,2-b]quinolin-9-yl-[1,4'bipiperidine]-1'-carboxylate), a camptothecin derivative topoisomerase I inhibitor.

[0055] MM-398 (see, e.g., US 8,147,867) is a stable liposomal formulation also known as irinotecan liposome injection, irinotecan sucrose octasulfate salt liposome injection, or irinotecan sucrosofate liposome injection (also referred to as PEP02, and ONIVYDE™). MM-398 may be provided as a sterile, injectable parenteral aqueous liquid for intravenous injection. The required amount of MM-398 may be diluted, e.g., in 250 mL of 5% dextrose injection USP and infused, e.g., over a 90 minute period.

[0056] An MM-398 liposome is a unilamellar lipid bilayer vesicle of 80-140 nm in diameter that encapsulates an aqueous space which contains irinotecan complexed in a gelated or precipitated state as a salt with sucrose octasulfate. The lipid membrane of the liposome is comprised of phosphatidylcholine, cholesterol, and a polyethyleneglycol-derivatized phosphatidyl-ethanolamine in the amount of approximately one polyethyleneglycol (PEG) molecule for 200 phospholipid molecules. This stable liposomal formulation of irinotecan has several attributes that provide an improved therapeutic index. The controlled and sustained release improves activity of the schedule-dependent drug irinotecan by increasing duration of exposure of tumor tissue to drug (either or both of irinotecan and its more active metabolite, SN-38), an attribute that allows drug to be present in a higher proportion of cells during the S-phase of the cell cycle, when DNA unwinding (mediated by topoisomerase) is required as a preliminary step in the DNA replication process.

[0057] The long circulating pharmacokinetics and high intravascular drug retention of liposomes (e.g., the MM-398 liposomes) can provide additional benefits, such as those stemming from enhanced permeability and retention (EPR). In tumors and certain other sites of pathology (e.g., sites of infection, inflammation, or both), the normal integrity of the vasculature (capillaries in

particular) is compromised, resulting in vascular permeability that allows leakage out of the capillary lumen of nano-particulates such as liposomes, which may then be retained and accumulate at the site of leakage. Such enhanced permeability and retention may thus promote preferential delivery and accumulation of liposomes within tumors and sites of inflammation or infection. EPR of MM-398 may result in a subsequent depot effect, where liposomes accumulate (e.g., in tumor associated macrophages), which can metabolize irinotecan, converting it locally to the substantially more cytotoxic SN-38) and release drug as they break down. This preferential local bioactivation and delivery is believed to result in increased exposure at cancer cells within the tumor and reduced drug exposure elsewhere (e.g., at potential sites of toxicity).

[0058] *IV. 5-Fluorouracil (5-FU) and Leucovorin.*

[0059] 5-Fluorouracil is a pyrimidine antagonist that interferes with nucleic acid biosynthesis. The deoxyribonucleotide of the drug inhibits thymidylate synthetase, thus inhibiting the formation of thymidylic acid from deoxyuridylic acid, thus interfering in the synthesis of DNA. It also interferes with RNA synthesis.

[0060] Leucovorin can potentiate the cytotoxic effects of fluorinated pyrimidines (e.g., 5-FU and floxuridine). For example, after 5-FU is activated within the cell, it becomes associated with a folate cofactor, and mediates cytotoxicity by inhibiting the enzyme thymidylate synthetase. Leucovorin can increase the folate pool, thereby increasing the binding of folate cofactor and active 5-FU with thymidylate synthetase, resulting in increased cytotoxicity.

[0061] *V. Bevacizumab*

[0062] Bevacizumab is a recombinant humanized monoclonal antibody that blocks angiogenesis by inhibiting vascular endothelial growth factor A (VEGF-A). VEGF-A is a cytokine that stimulates angiogenesis in a variety of diseases, especially in cancer. Bevacizumab is administered at a dose of 5 mg/kg, or 10 mg/kg typically Q2W, or 15 mg/kg, typically Q3W.

[0063] *VI. Administration*

[0064] Liposomal irinotecan is administered intravenously in combination with co-administration of free irinotecan, and optionally in further combination with co-administration of 5-fluorouracil (5-FU), and leucovorin, which optionally are co-administered in further combination with bevacizumab. In one embodiment, free irinotecan is administered prior to liposomal irinotecan. In another embodiment, free irinotecan and liposomal irinotecan are administered prior to 5-FU and leucovorin. In another embodiment, leucovorin is administered prior to 5-FU. In another embodiment, bevacizumab is administered prior to liposomal irinotecan, free irinotecan, leucovorin, and 5-fluorouracil. In another embodiment, liposomal irinotecan is administered intravenously over 60 minutes or 90 minutes. In another embodiment

free irinotecan is administered intravenously over 60 minutes. In another embodiment, 5-FU is administered intravenously over 46 hours. In another embodiment, leucovorin is administered intravenously over 2 hours. In another embodiment bevacizumab and leucovorin are each administered over 120 minutes. In another embodiment bevacizumab is administered over 30-90 minutes. In various embodiments the liposomal irinotecan is MM-398.

[0065] VII. Patient Populations

[0066] The compositions and methods disclosed herein are useful for the treatment of patients with a variety cancers including unresectable cancers and cancers that are refractory or resistant to other anti-cancer treatments.

[0067] CRC

[0068] In one embodiment, a patient treated using the methods and compositions disclosed herein has histologically proven carcinoma.

[0069] The patient treated may have advanced or metastatic disease not suitable for complete surgical resection (*e.g.*, unresectable cancer).

[0070] The patient treated may have colorectal cancer (CRC).

[0071] The patient treated may have unresectable or metastatic CRC.

[0072] The CRC may be adenocarcinoma, squamous cell carcinoma, leiomyosarcoma, carcinoid tumor, or gastrointestinal stromal tumor.

[0073] In various of the CRC embodiments, the patient has at least one, or all, of:

[0074] a. histologically proven carcinoma,

[0075] b. documented advanced or metastatic disease not suitable for complete surgical resection

[0076] c. Measurable lesions according to RECIST v1.1 criteria

[0077] d. ECOG performance status 0 – 1

[0078] e. bone marrow reserves as evidenced by:

[0079] • ANC $\geq 1.5 \times 10^9$/L without the use of hematopoietic growth factor

[0080] • platelets $\geq 100 \times 10^9$/L

[0081] • hemoglobin > 9 g/dL (may be transfused to maintain or exceed this level)

[0082] f. International Normalized Ratio (INR) ≤1.5; aPTT<1.5 x UNL; EXEPT THAT: patients on full anticoagulation due to VTE must have an in-range INR (between 2 and 3).

[0083] g. adequate renal function as evidenced by:

[0084] • serum creatinine: < 150 μmol/l

[0085] • calculated creatinine clearance >50ml/min. (recommendation: to be calculated according to the MDRD formula)

[0086] h. total bilirubin <1.0 x upper normal limit (ULN), or

[0087] i. normal ECG, or ECG without any clinically significant findings.

[0088] In various of the CRC embodiments, the patient does not have one or more of:

[0089] a. Active central nervous system metastases (e.g., indicated by clinical symptoms, cerebral edema, steroid requirement, or progressive disease)

[0090] b. Bone-only disease

[0091] c. Clinically significant gastrointestinal disorder (other than CRC) including hepatic disorders, bleeding, inflammation, GI obstruction, or diarrhea > grade 1

[0092] d. Patients refractory to irinotecan (i.e. prior exposure to irinotecan-based therapy with progressive disease as best response)

[0093] e. Known DLT responses to irinotecan

[0094] f. Patients known to be homozygous for UGT1A1 *28

[0095] g. History of any second malignancy in the last 3 years; patients with prior history of in-situ cancer or basal or squamous cell skin cancer are eligible. Patients with a history of other malignancies are eligible if they have been continuously disease-free for at least 3 years

[0096] h. Prior exposure to MM-398

[0097] i. Known hypersensitivity to any of the components of MM-398, or other liposomal products

[0098] j. Concurrent illnesses that would be a relative contraindication to trial participation such as active cardiac or liver disease

[0099] • Severe arterial thromboembolic events (myocardial infarction, unstable angina pectoris, stroke) less than 6 months before inclusion

[00100] • NYHA Class III or IV congestive heart failure, ventricular arrhythmias

[00101] k. Active infection or an unexplained fever >38.5°C during screening visits or on the first scheduled day of dosing (at the discretion of the investigator, patients with tumor fever may be enrolled), which in the investigator's opinion might compromise the patient's participation in the trial or affect the study outcome

[00102] l. Prior chemotherapy administered within 3 weeks, or within a time interval less than at least 5 half-lives of the agent, whichever is longer, prior to the first scheduled day of dosing in this study

[00103] m. Uncontrolled hypertension (defined as persistent systolic blood pressure >150 mmHg and/or diastolic blood pressure >100 mmHg), or history of hypertensive crisis, or hypertensive encephalopathy

[00104] n. Received radiation therapy in the last 14 days

[00105] o. Major surgery or traumatic injury within the last 28 days

[00106] p. Any other medical or social condition deemed by the Investigator to be likely to interfere with a patient's ability to sign informed consent, cooperate and participate in the study, or interfere with the interpretation of the results

[00107] q. Pregnant or breast feeding; females of child-bearing potential must test negative for pregnancy at the time of enrollment based on a urine or serum pregnancy test. Both male and female patients of reproductive potential must agree to use a reliable method of birth control, during the study and for3 months following the last dose of study drug.

[00108] r. Concomitant administration use with St John Worth,

[00109] s. Concomitant administration of live attenuated virus vaccine such as yellow fever vaccine

[00110] In other embodiments, the patient has a pancreatic cancer, e.g., an exocrine pancreatic cancer. In one such embodiment, the pancreatic cancer selected from the group consisting of acinar cell carcinoma, adenocarcinoma, adenosquamous carcinoma, giant cell tumor, intraductal papillary-mucinous neoplasm (IPMN), mucinous cystadenocarcinoma, pancreatoblastoma, serous cystadenocarcinoma, and solid and pseudopapillary tumors. A patient treated may have recurrent or persistent pancreatic cancer following primary chemotherapy. The patient with pancreatic cancer may have had previously been treated with and failed at least one platinum-based chemotherapy regimen for management of primary or recurrent disease, *e.g.*, a chemotherapy regimen comprising oxaliplatin, carboplatin, cisplatin, or another organoplatinum compound. Alternately, the patient with pancreatic cancer may have failed prior treatment with gemcitabine or become resistant to gemcitabine.

[00111] *VIII. Combination Therapy*

[00112] According to this invention, a drug in liposomally encapsulated form and the same drug in unencapsulated and unmodified (free) form are co-administered to a patient in need thereof. Such co-administration improves the pharmacokinetic profile of the drug in the patient. The patient may have cancer. The drug may be irinotecan.

[00113] In one embodiment, liposomal irinotecan is co-administered with free irinotecan to patients having cancer, according to a particular clinical dosage regimen, such as those described herein. In another embodiment, the patients have unresectable cancer. In another

embodiment, the patients have colon cancer. In another embodiment, the patients have unresectable colon cancer.

[00114] In another embodiment, liposomal irinotecan is co-administered to a cancer patient in combination with free irinotecan, 5-fluorouracil (5-FU), leucovorin, and (optionally) bevacizumab. Administration of each of these combinations is to a patient having cancer, according to a particular clinical dosage regimen, such as those described herein. The liposomal irinotecan may be MM-398. The patient may have unresectable cancer. The patient may have CRC. The CRC may be unresectable. The patient may have metastatic cancer. The patient may have metastatic CRCr. The patient may have unresectable metastatic CRC.

[00115] Liposomal irinotecan may be co-administered to patients having pancreatic cancer in combination with free irinotecan, 5-fluorouracil (5-FU) and leucovorin, according to a particular clinical dosage regimen, such as those described herein.

[00116] In all of the foregoing embodiments, an exemplary liposomal irinotecan is MM-398. Liposomal irinotecan can be simultaneously or sequentially administered with free irinotecan. Alternatively, liposomal irinotecan can be co-administered with free irinotecan, 5-FU and leucovorin, wherein liposomal irinotecan, 5-FU and leucovorin are each formulated for separate administration and are administered sequentially. For example, liposomal and free irinotecan can be administered first followed by (e.g., immediately followed by) the administration of the leucovorin and then 5-FU. In another embodiment, free irinotecan and liposomal irinotecan are administered prior to 5-FU, leucovorin, and bevacizumab. In another embodiment, bevacizumab is administered prior to liposomal irinotecan, free irinotecan, leucovorin, and 5-fluorouracil.

[00117] Liposomal irinotecan, free irinotecan, 5-FU, leucovorin, and bevacizumab may each be separately formulated for intravenous administration. In an exemplary embodiment, the patient is administered effective therapy comprising administration of each of liposomal irinotecan, free irinotecan, 5-fluorouracil (5-FU), and leucovorin, wherein the treatment comprises at least one cycle, wherein the cycle is a period of 2 weeks, and wherein for each cycle: (a) liposomal irinotecan is administered at a dose of 60 or 80 mg/m^2; (b) free irinotecan is administered at a dose of 90 or 120 mg/m^2; (c) leucovorin is administered at a dose of 400 mg/m^2; and (d) 5-fluorouracil is administered at a dose of 2400 mg/m^2. The therapy may further comprise administration of bevacizumab at a dose of 5 mg/kg or 10 mg/kg.

[00118] Alternately, the patient is administered effective therapy comprising administration of each of liposomal irinotecan and free irinotecan without administration of leucovorin or 5-FU, and optionally without administration of bevacizumab, wherein the

treatment comprises at least one cycle, wherein the cycle is a period of 2 weeks, and wherein for each cycle: (a) liposomal irinotecan is administered at a dose of 60 or 80 mg/m^2; (b) free irinotecan is administered at a dose of 90 or 120 mg/m^2.

[00119] *IX. Outcomes*

[00120] Preferably, co-administration of liposomal irinotecan and free irinotecan exhibits therapeutic synergy, or the co-administration of liposomal irinotecan, free irinotecan, 5-fluorouracil (5-FU) and leucovorin exhibits therapeutic synergy, or the co-administration of liposomal irinotecan, free irinotecan, 5-fluorouracil (5-FU), leucovorin and bevacizumab exhibits therapeutic synergy.

[00121] Such co-administration of these combinations produces an additive or superadditive effect on suppressing tumor growth, as compared to monotherapy with liposome-encapsulated irinotecan alone or treatment with the other preparations in the absence of liposomal irinotecan therapy. By "additive" is meant a result that is greater in extent than the best separate result achieved by monotherapy with each individual component, while "superadditive" is used to indicate a result that exceeds in extent the sum of such separate results. In one embodiment, the additive effect is measured as slowing or stopping of tumor growth. The additive effect can also be measured as, e.g., reduction in size of a tumor, reduction of tumor mitotic index, reduction in number of metastatic lesions over time, increase in overall response rate, or increase in median or overall survival or in the frequency and/or duration of symptom-free or symptom-reduced periods.

[00122] One non-limiting example of a measure by which effectiveness of a therapeutic treatment can be quantified is by calculating the log10 cell kill, which is determined according to the following equation:

[00123] log10 cell kill = T C (days)/3.32 × Td

[00124] in which T C represents the delay in growth of the cells, which is the average time, in days, for the tumors of the treated group (T) and the tumors of the control group (C) to have reached a predetermined value (1 g, or 10 mL, for example), and Td represents the time, in days necessary for the volume of the tumor to double in the control animals. When applying this measure, a product is considered to be active if log10 cell kill is greater than or equal to 0.7 and a product is considered to be very active if log10 cell kill is greater than 2.8. Using this measure, a combination, used at its own maximum tolerated dose, in which each of the constituents is present at a dose generally less than or equal to its maximum tolerated dose, exhibits therapeutic synergy when the log10 cell kill is greater than the value of the log10 cell kill of the best constituent when it is administered alone. In an exemplary case, the log10 cell kill of the

combination exceeds the value of the log10 cell kill of the best constituent of the combination by at least 0.1 log cell kill, at least 0.5 log cell kill, or at least 1.0 log cell kill.

[00125] Responses to therapy may include:

[00126] Pathologic complete response (pCR): absence of invasive cancer in the breast and lymph nodes following primary systemic treatment.

[00127] Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) which has reduction in short axis to <10 mm;

[00128] Partial Response (PR): At least a 30% decrease in the sum of dimensions of target lesions, taking as reference the baseline sum diameters;

[00129] Stable Disease (SD): Neither sufficient shrinkage to qualify for partial response, nor sufficient increase to qualify for progressive disease, taking as reference the smallest sum diameters while on study; or

[00130] Meanwhile, non-CR/Non-PD denotes a persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

[00131] Progressive Disease (PD) denotes at least a 20% increase in the sum of dimensions of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of 5 mm. The appearance of one or more new lesions is also considered progression;

[00132] In exemplary outcomes, patients treated according to the methods disclosed herein may experience improvement in at least one sign of cancer.

[00133] In one embodiment the patient so treated exhibits pCR, CR, PR, or SD.

[00134] In another embodiment, the patient so treated exhibits tumor shrinkage and/or decrease in growth rate, i.e., suppression of tumor growth. In another embodiment, tumor cell proliferation is reduced or inhibited. In yet another embodiment, one or more of the following can occur: the number of cancer cells can be reduced; tumor size can be reduced; cancer cell infiltration into peripheral organs can be inhibited, retarded, slowed, or stopped; tumor metastasis can be slowed or inhibited; tumor growth can be inhibited; recurrence of tumor can be prevented or delayed; one or more of the symptoms associated with cancer can be relieved to some extent.

[00135] In other embodiments, such improvement is measured by a reduction in the quantity and/or size of measurable tumor lesions.

[00136] In some embodiments, co-administration of effective amounts of liposomal irinotecan and free irinotecan according to any of the methods provided herein produce at least one therapeutic effect selected from the group consisting of reduction in size of a tumor,

reduction in number of metastatic lesions appearing over time, complete remission, partial remission, stable disease, increase in overall response rate, or a pathologic complete response. In some embodiments, the provided methods of treatment produce a comparable clinical benefit rate (CBR = CR+ PR+ SD ≥ 6 months) better than that achieved by the same combinations of anti-cancer agents administered without concomitant MM-398 administration. In other embodiments, the improvement of clinical benefit rate is about 20% 20%, 30%, 40%, 50%, 60%, 70%, 80% or more compared to the same combinations of anti-cancer agents administered without concomitant MM-398 administration.

[00137] In other embodiments, co-administration of effective amounts of liposomal irinotecan, free irinotecan, 5-FU, leucovorin, and bevacizumab according to any of the methods provided herein produce at least one therapeutic effect selected from the group consisting of reduction in size of a tumor, reduction in size of a unresectable colorectal tumor, reduction in number of metastatic lesions appearing over time, complete remission, partial remission, stable disease, increase in overall response rate, or a pathologic complete response. In some embodiments, the provided methods of treatment produce a comparable clinical benefit rate (CBR = CR+ PR+ SD ≥ 6 months) better than that achieved by the same combinations of anti-cancer agents administered without concomitant MM-398 administration. In other embodiments, the improvement of clinical benefit rate is about 20% 20%, 30%, 40%, 50%, 60%, 70%, 80% or more compared to the same combinations of anti-cancer agents administered without concomitant MM-398 administration.

[00138] The following examples are illustrative and should not be construed as limiting the scope of this disclosure in any way; many variations and equivalents will become apparent to those skilled in the art upon reading the present disclosure.

[00139] **EXAMPLES**

[00140] Example 1: MM-398 Pre-Clinical Pharmacokinetics

[00141] Pharmacokinetic properties of free irinotecan and MM-398 were evaluated in an HT-29 colon subcutaneous xenograft model. Tumor bearing mice were injected with different doses of MM-398 (5, 10, 20mg/kg) and following a single injection, plasma and tissue samples were collected at various time points (1, 4, 8, 24, 48, 72, 168hours). HPLC analysis was used to measure the levels of the irinotecan and its metabolite SN-38 in these samples. The PK profile of MM-398 was compared with that of free irinotecan (at 10 and 40mg/kg).

[00142] Both irinotecan and SN-38 are cleared very rapidly (within 8 hours) from the plasma following free irinotecan administration. However, MM-398 clearance is considerably slower with a half-life of approximately 48 hours as shown in Figure 1A; as >90% of irinotecan

is encapsulated throughout in the plasma, irinotecan levels are reflective of MM-398 concentration. SN38 plasma exposure is also greater though Cmax levels are reduced following MM-398 administration, suggesting the advantage of the irinotecan liposomal formulation in prolonging exposure and half-life (Figure 1B). Both irinotecan and SN-38 accumulate in tissues for extended time (at least 1 week after MM-398 administration). Also, the accumulation was observed to be dose-dependent (Figure 1C).

[00143] Activation of irinotecan to SN38 by the liver is the primary path for SN38 tumoral accumulation when free irinotecan is administered. In contrast, these data suggest that accumulation of MM-398 in the tumor and subsequent liposome breakdown and local conversion of irinotecan to SN38 is responsible for the enhanced tumor exposure of SN38 when MM-398 is administered. Current research is focused on identifying cell types located in the tumor responsible for liposomal breakdown and activation of MM-398.

[00144] **Example 2:** A Phase I study of MM-398 plus irinotecan in unresectable advanced cancer.

[00145] ***Objectives***

[00146] The primary objective of this study is to determine the safety, tolerability and range of tolerated combination doses, and to define the recommended dose (RD) for further evaluation in a phase II study. 1) To determine the safety, tolerability and range of tolerated combination dose. 2) To define the recommended dose (RD) for further evaluation in a phase II study.

[00147] The secondary objectives of this study are: To describe the pharmacokinetics of MM-398 plus irinotecan combination therapy (group A). To describe the pharmacokinetics of MM-398 plus irinotecan and LV/5-FU combination therapy (group B). To determine the clinical activity of MM-398 plus irinotecan combination therapy (group A). To determine the clinical activity of MM-398 plus irinotecan and LV/5-FU combination therapy (group B).

[00148] Exploratory objectives of this study are to evaluate the pharmacodynamic response in colorectal tumors following combination therapy, correlations between potential tissue and plasma/serum pharmacodynamic markers (Tumor Associated Macrophages (TAM), tumor irinotecan, tumor SN-38, tumor SN38G levels, plasma cytokines, plasma apolipoproteins or other plasma components) and tumor response, drug clearance and safety.

[00149] ***Trial Design***

[00150] This is a dose-escalation and therapeutic exploratory phase I multi-center, open label study of MM-398 plus irinotecan patients with unresectable advanced cancer.

[00151] This study will enroll approximately 6-36 patients in two groups.

[00152] ▪ Group A, patients with unresectable advanced non-colorectal cancer who would receive MM-398 and free irinotecan every two weeks (q2w).

[00153] ▪ Group B, patients with metastatic colorectal cancer who would receive MM-398 and free irinotecan co-administered with bevacizumab 5mg/kg, leucovorin 400 mg/m^2 2 h infusion and 5-Fluorouracil 2400 mg/m^2 46-h infusion, every two weeks.

[00154] There are three periods to this study: ***1) Screening Period*** (up to 28 days): Patients undergo screening assessments to determine the eligibility for the study. ***2) MM-398 treatment Period*** (C1D1 until intolerable toxicity and/or progression): Patients receive treatment every 2 weeks and undergo biopsies and other required assessments. ***3) Follow-up Period*** Patients are assessed 30 days after their last dose of MM-398 for final safety assessments, and every 2 months thereafter for overall survival follow-up.

[00155] ***Dose-Limiting Toxicity***

[00156] Dose-limiting toxicities (DLT) are defined as any of the following events that are possibly, probably or definitely attributable to the combination of MM-398 and irinotecan, and are considered to be clinically significant. DLTs for the purposes of dose-escalation are evaluated during a 28-day period following the first dose of the study treatment. This includes the two treatment cycles. Each treatment cycle is 2 weeks. Toxicities are graded and documented according the NCI CTCAE (v4.0).

[00157] Non-hematological dose-limiting toxicity is defined as:

[00158] ▪ Any Grade 3 or Grade 4 non-hematological toxicity with the specific exclusion of:

[00159] ○ Grade 3 nausea, vomiting, diarrhea, mucositis/stomatitis that responds to maximal supportive treatment(s) within 3 days;

[00160] ○ Grade 3 liver enzyme elevation, including ALT/AST/GGT that returns to Grade ≤1 or baseline prior to the time for the next treatment cycle; and

[00161] ○ Grade 3 fever or infection

[00162] ▪ Allergic reactions that necessitate discontinuation of study drug.

[00163] Hematological dose-limiting toxicity is defined as:

[00164] ▪ Grade 4 neutropenia (absolute neutrophil cont, ANC) for > 7 days Platelet count <10.000/mm^3 on 2 separate days or requiring a platelet transfusion on 2 separate days within a 7 day period

[00165] ▪ Toxicity that causes a delay of >14 days between treatment cycles.

[00166] ▪ Febrile neutropenia despite G-CSF secondary prophylaxis

[00167] ***Dose Escalation and Definition of Maximum Tolerated Dose***

[00168] The objective of the dose escalation is to define, with a limited number of patients, the safety and toxicity characteristics of MM-398 given with free irinotecan. Dose escalation will follow a 3+3 dose escalation procedure as described in Table 4. Evaluation of safety data format least 3 patients who have completed 28 days of dosing on study is required prior to defining a new dose and starting the next cohort. After the last patient in each cohort completes the DLT evaluation period, the safety data, including labs, all adverse events (AEs) and any other relevant data collected, is assessed. Dosing will proceed to the next level after agreement between the Investigators, Sponsor and Medical Monitor. Patients are enrolled in cohorts of 3 for each dose level. Dose escalation proceeds between each cohort and no intra-patient dose escalation is allowed. If none of the first 3 patients experiences DLT, then dose escalation proceeds for the next cohort of patients. If 1 of 3 patients develop DLT, the cohort is expanded to 6 patients. If no more than 1 of the 6 patients experience DLT, then escalation to the next dose level occurs. If 2 of 3 or 2 of 6 patients develop DLT at a certain dose level, the dose escalation is withheld and the prior dose level is verified as maximum tolerated dose (MTD). A minimum of 6 evaluable patients are treated at the MTD dose level and no more than 1 of the 6 patients should experience DLT at this dose level. If the highest dose level is evaluated without a DLT in the initial 3 patients, than an additional 3 patients are enrolled. If no more than 1 of 6 patients experience DLT at the highest dose level, than this is declared the MTD.

[00169] **Table 1: Dose Escalation Decision Rules**

Number of Patients with DLT at a Given Dose Level	Action
0 out of 3	Enter 3 patients at the next dose level
1 out of 3	Enter at least 3 more patients at this dose level. • If 0 of these 3 patients experiences a DLT (i.e., no more than 1 of 6 for the entire cohort), dose escalation proceeds to the next dose level. • If 1 or more patients of this group suffers a DLT (i.e., ≥ 2 of 6 for the entire cohort), then dose escalation is stopped, and this dose is declared to have exceeded the MTD. Three (3) additional patients are entered at the next lowest dose level if only 3 patients were treated previously at that dose.

≥ 2 of 3	Dose escalation is stopped. This dose level is declared to have exceeded the MTD. Three (3) additional patients are entered at the next lowest dose level if only 3 patients were treated previously at that dose.
Highest dose level producing ≤ 1 DLT out of 6 patients	This is the MTD and is generally the recommended stage 2 dose. At least 6 patients must be entered at the recommended dose level.

[00170] The final MTD after sequential MM-398 and CPT11 dose escalations will be considered as the recommended dose for the Phase II study. Patients who experience a DLT are discontinued from the study. Patients who are non-compliant with study procedures, or discontinue from the study during the DLT evaluation period for reasons other than toxicity, are replaced.

[00171] **Efficacy**

[00172] Tumor responses are evaluated every 8 weeks after start of treatment. The modified Response Evaluation Criteria in Solid tumors (RECIST version 1.1; Eisenhauer, E. A., *et al.* (2009), EJC, 45(2), 228–47) is for this study for objective tumor response assessment, including confirmation of response within 28 days. The best overall response is the best response recorded from the start of treatment until treatment failure, taking as reference for progressive disease the smallest measurements recorded since the treatment started. Survival is assessed from the date of randomization to the date of patient death, due to any cause, or to the last date the patient was known to be alive. Patients who were not reported as having died at the time of the analysis are censored using the date they were last known to be alive. PFS (progression-free survival) is the time from the date of first dose of study drug to the date of progressive disease (RECIST criteria) or death (any cause). Death is regarded as a progression event in those patients. Patients without documented objective progression at the time of the final analysis are censored at the date of their last objective tumor assessment.

[00173] **Patient population**

[00174] Patients must fulfill all of the following criteria to be eligible for the study.

[00175] *Inclusion criteria:*

[00176] a. Age 18 – 75 years

[00177] b. Histologically proven carcinoma

[00178] c. Documented advanced or metastatic disease not suitable for complete surgical resection

[00179] d. Measurable lesions according to RECIST v1.1 criteria

[00180] e. ECOG performance status 0 – 1

[00181] f. Bone marrow reserves as evidenced by:

[00182] * ANC ≥ 1.5 x 10^9/L without the use of hematopoietic growth factors

[00183] * platelets ≥ 100 x 10^9/L

[00184] * hemoglobin > 9 g/dL (may be transfused to maintain or exceed this level)

[00185] g. International Normalized Ratio (INR) ≤ 1.5; aPTT <1.5 x UNL; EXEMPTION: patients on full anticoagulation due to VTE must have an in-range INR (usually between 2 and 3).

[00186] h. Adequate renal function as evidenced by:

[00187] * serum creatinine: < 150 µmol/l

[00188] * calculated creatinine clearance > 50ml/min. (recommendation: to be calculated according to the MDRD formula)

[00189] i. total bilirubin < 1.0 x upper normal limit (ULN)

[00190] j. Normal ECG or ECG without any clinically significant findings

[00191] k. Regular follow-up feasible. A registered patient must be treated and followed at the participating center.

[00192] l. Able to understand and sign an informed consent (or have a legal representative who is able to do so)

[00193] m. Registration in a national health care system (CMU included for France).

[00194] *Exclusion Criteria:*

[00195] a. Active central nervous system metastases (indicated by clinical symptoms, cerebral edema, steroid requirement, or progressive disease)

[00196] b. Bone-only disease

[00197] c. Clinically significant gastrointestinal disorder including hepatic disorders, bleeding, inflammation, GI obstruction, or diarrhea > grade 1

[00198] d. Patients refractory to irinotecan (i.e. prior exposure to irinotecan-based therapy with progressive disease as best response)

[00199] e. Known DLT responses to irinotecan

[00200] f. Patients known to be homozygous for UGT1A1 *28

[00201] g. History of any second malignancy in the last 3 years; patients with prior history of in-situ cancer or basal or squamous cell skin cancer are eligible. Patients with a

history of other malignancies are eligible if they have been continuously disease-free for at least 3 years

[00202] h. Prior exposure to MM-398

[00203] i. Known hypersensitivity to any of the components of MM-398, or other liposomal products

[00204] j. Concurrent illnesses that would be a relative contraindication to trial participation, such as active cardiac or liver disease

[00205] ⊗ Severe arterial thromboembolic events (myocardial infarction, unstable angina pectoris, stroke) less than 6 months before inclusion

[00206] ⊗ NYHA Class III or IV congestive heart failure, ventricular arrhythmias

[00207] k. Active infection or an unexplained fever > 38.5°C during screening visits or on the first scheduled day of dosing (at the discretion of the investigator, patients with tumor fever may be enrolled), which in the investigator's opinion might compromise the patient's participation in the trial or affect the study outcome

[00208] l. Prior chemotherapy administered within 3 weeks, or within a time interval less than at least 5 half-lives of the agent, whichever is longer, prior to the first scheduled day of dosing in this study

[00209] m. Uncontrolled hypertension (defined as persistent systolic blood pressure >150 mmHg and/or diastolic blood pressure >100 mmHg), or history of hypertensive crisis, or hypertensive encephalopathy

[00210] n. Received radiation therapy in the last 14 days

[00211] o. Major surgery or traumatic injury within the last 28 days

[00212] p. Any other medical or social condition deemed by the Investigator to be likely to interfere with a patient's ability to sign informed consent, cooperate and participate in the study, or interfere with the interpretation of the results

[00213] q. Pregnant or breast feeding; females of child-bearing potential must test negative for pregnancy at the time of enrollment based on a urine or serum pregnancy test. Both male and female patients of reproductive potential must agree to use a reliable method of birth control, during the study and for 3 months following the last dose of study drug.

[00214] r. Concomitant administration use with St John Worth

[00215] s. Concomitant administration of live attenuated virus vaccine such as yellow fever vaccine

[00216] Study Treatment Discontinuation

[00217] Patients may withdraw or be withdrawn from the study at any time and for any reason. Some possible reasons for early withdrawal include, but are not limited to, the following: Progressive neoplastic disease. An adverse event which precludes further participation. Development of an intercurrent medical condition or need for concomitant treatment that precludes further participation. Noncompliance with the protocol. Patient withdraws consent. Investigator removes the patient from the trial in the best interest of the patient. Study termination by the Sponsor. Use of prohibited concomitant medications. Lost to follow up.

[00218] All procedures and evaluations required by the 30 day follow up visit are completed when a patient is discontinued from treatment, and the patients should continue to be followed-up for overall survival. All patients who discontinue the trial as a result of an adverse event must be followed until resolution or stabilization of the adverse event.

[00219] **Treatment Period**

[00220] The treatment phase consists of 5 dose levels. A "3 + 3" design is utilized. Patients will be treated until disease progression or unacceptable toxicity. Each dose cohort starts with 3 patients. DLT evaluation period is during cycle 1 and 2 (28-days period). **Irinotecan** dose levels 60 or 80 mg/m^2 diluted in 250 ml NaCl 0.9%, 1 hour infusion. **MM-398** dose levels 90 or 120 mg/m^2 diluted in 250 ml dextrose 5%, 90 minute infusion. The MM-398 infusion time may be reduced to 1 hour starting with cycle 2 if no acute infusion reaction has occurred in cycle 1. Group B, patients with metastatic colorectal cancer, will receive q2w treatment with leucovorin 400 mg/m^2, 2hr infusion, 5-fuorouracil 2400 mg/m^2, 46hr infusion and bevacizumab 5mg 30-90 minute infusion, in addition to irinotecan and MM-398.

[00221] **Schedule and Dose Modifications**

[00222] Specific dose modifications for irinotecan/MM-398

[00223] Patients are discontinued from study treatment if they:

[00224] • Experience a DLT

[00225] • Experience any of the toxicities defined below that do not resolve to baseline within 14 days after the planned start of next treatment cycle

[00226] • Require more than one dose reduction

[00227] Patients who meet the above criteria are allowed to continue in the study if the patient is receiving clinical benefit. Once a dose has been reduced, it cannot be escalated back to the previous level. If Grade 3 (> 3 days) or Grade 4 therapy-associated diarrhea is experienced by a patient despite maximal use of anti-diarrheal medications, the dose of irinotecan/MM-398 is reduced to the next lower dose level as defined in stage 1 for subsequent cycles. If Grade 3 (> 3 days) or Grade 4 diarrhea is documented despite maximal use of anti-diarrheal drugs,

prophylactic antibiotics, and the dose reductions, the patient comes off protocol therapy. Patients who have Grade 3 or 4 thrombocytopenia receive subsequent cycles at the next lower dose level. Retreatment at these lower doses is initiated when thrombocytopenia has normalized to Grade 2 or better. Patients who have Grade 4 neutropenia of > 7 days duration or febrile neutropenia receive subsequent cycles at the next lower dose level and may receive prophylactic myeloid growth factor treatment. Retreatment at these lower doses is initiated when the neutropenia has normalized to Grade 2 or better. For non-hematological toxicity, that meets the definition of a DLT, other than those specifically listed above, subsequent cycles of therapy is administered at the next lower dose level.

[00228] *Management of Infusion Reactions*

[00229] Therapy modifications for patients who develop infusional reactions to MM-398 follow procedures outlined in Table 5:

[00230] **Table 2: Therapy Modification After Infusion Reactions to MM-398**

Grade	Action
Grade 1 Transient flushing or rash, drug fever < 38◦ C (<100.4 ◦ F) and **Grade 2** Rash, flushing, urticaria, dyspnea, drug fever ≥ 38◦ C (≥100.4 ◦ F)	○ If a patient develops a hypersensitivity reaction despite diphenhydramine pretreatment, stop the infusion and wait 30 to 60 minutes (depending upon the reaction severity). At the physician's discretion, it may be possible to resume treatment by administering an H2 blocker approximately 30 minutes before restarting the infusion. For H2 receptors antagonists, use the manufacturer recommends famotidine 0.5 mg/kg IV maximum dose 20 mg, rather than cimetidine, because it lacks reported drug interactions. If famotidine is unavailable, administer ranitidine 1-2 mg/kg IV maximum dose 50 mg. Re-attempt infusion at a slower rate, possibly over 120 minutes. ○ If grade 1-2 infusion reactions recur with subsequent dose, add dexamethasone 0.2 mg/kg (max 10mg) IV or equivalent to premedications above. (Only dose interruption/discontinuation, but not dose reduction is required for allergic/infusional reactions)
Grade 3 Symptomatic bronchospasm with or	○ Stop infusion immediately and remove the infusion tube ○ Administer diphenhydramine hydrochloride 1 mg/kg IV (max 50 mg), dexamethasone 0.2mg/kg (max 10mg) IV (or

without urticaria, allergy-related edema/angioedema, hypotension	equivalent), bronchodilators for bronchospasms, and other medications as medically indicated. Hospital admission is considered. o Discontinue MM-398 treatment
Grade 4 Anaphylaxis	o Stop infusion immediately and remove the infusion tube o Administer diphenhydramine hydrochloride 1 mg/kg (max 50mg) IV, dexamethasone 0.2 mg/kg (max 10mg) IV (or equivalent), and other anaphylaxis medications as indicated. Epinephrine or bronchodilators should be administered as indicated. Hospital admission for observation may be indicated o Discontinue MM-398 treatment

[00231] ***Dose adaptation for 5-fluorouracil (Group B)***

Type of Toxicity **NCI CTC Grade**	**5-FU C. Infusion. Doses (mg/m²/course)** **NCI CTC Grade**
Hemoglobin (any grade)	none
Neutrophils Gr 3-4 and/or Platelets Gr 2-4	2000
Nausea and/or Vomiting Grade 4 despite pre-medication	2000
Diarrhea Grade 3-4	2000
Stomatitis Grade 3-4	2000
Heart> Grade 1	Stop treatment
Skin Grade 3 or 4	2000
Allergy	Contact the medical coordinator for specific case
Neurocerebellar	Stop treatment
Local Tolerance (any Grade)	none
Alopecia (any Grade)	none
Other Toxicity clearly drug related: - Grade 1 and 2 - Grade 3 - Grade 4	 none 2000 Stop

[00232] *Dose adaptation for bevacizumab (Group B)*

[00233] Dose of bevacizumab will not be reduced nor escalated. If serious bevacizumab related toxicity, bevacizumab will be suspended temporarily or definitively. Any grade 3 toxicity attributable to Bevacizumab will require treatment with Bevacizumab to be modified or discontinued. If toxicity resolves to ≤ grade 1 within 4 weeks, treatment will be restarted (on scheduled days). No dose reductions of Bevacizumab are permitted. Missed doses of Bevacizumab and combination chemotherapy will not be made up. Any patient who develops any one of the following toxicities attributable to Bevacizumab should not receive further Bevacizumab: Grade 4 toxicity; Grade 3 toxicity that does not resolve to grade 1 or less within 4 weeks; Arterial thromboembolic events; Gastrointestinal perforation. The schedule of study drug administration will be modified in the event of certain grades of adverse events, as summarized in the following Table.

[00234] *Table 3: Dose Modification of Bevacizumab*

Event	Action to Be Taken
Grade 3 or 4 thrombosis/embolism	**All toxicity will be graded according to NCI-CTC guidelines. Patients who develop Grade 3 or 4 thrombosis/embolism must discontinue Bevacizumab and the following action is recommended:** ▪ **Bevacizumab should be permanently discontinued in patients who develop arterial thromboembolic events.** ▪ **Grade 3 or 4 thrombosis: Hold Bevacizumab treatment for 2 weeks and then may be resumed during the period of therapeutic-dose anticoagulant therapy as soon as all of the following criteria are met:** o **The patient must be on a stable dose of anticoagulant and, if on warfarin, have an INR within the target range (usually between 2 and 3) prior to restarting study drug treatment** o **The patient must not have had a Grade 3 or 4 haemorrhagic event since entering the study** o **The patient must not have had any evidence of tumour invading or abutting major blood vessels on any prior CT scan.** ▪ **Symptomatic Grade 4 thrombosis: Discontinue Bevacizumab**
Hemorrhage	Grade 1 and 2: No dose modification Grade 3 or 4 (**first occurrence**): **Discontinue Bevacizumab.**

Event	Action to Be Taken
Proteinuria *	**First occurrence of proteinuria:** Adjustment of Bevacizumab administration for proteinuria will occur according to the following guidelines: ○ **<2+ (dipstick)**: Administer Bevacizumab as scheduled, NO additional evaluation is required. ○ **2+ , 3+ and 4+ proteinuria (dipstick)**: Administer Bevacizumab as scheduled and collect 24-hour urine to determine the total protein within 3 days prior to the next scheduled dose: * 24-hour proteinuria ≤2g: Administer Bevacizumab as scheduled. Repeat dipstick is required before each scheduled administration of Bevacizumab. * 24-hour proteinuria >2g: Bevacizumab treatment should be withheld pending next 24 hour total protein. - Repeat 24-hour urine protein ≤2g: Administer Bevacizumab as schedule. 24-hour protein should be further monitored prior to each administration of Bevacizumab until it has decreased to ≤1g/24-hour. - Repeat 24-hour urine protein >2g: Bevacizumab dose should be withheld until 24-hour protein has decreased to ≤2g. 24-hour protein should be further monitored prior to each administration of Bevacizumab until it has decreased to ≤1g/24-hour. **Second and subsequent occurrence of < 3+ proteinuria (dipstick):** Administer Bevacizumab as scheduled and no further 24-hour urine protein is required.
Hypersensitivity reaction Hypersensitivity reaction attributable to Bevacizumab grade 3 or grade 4	* Treat patient (antihistaminics, corticosteroids) * Discontinue Bevacizumab
Gastrointestinal perforation Gastrointestinal perforation or dehiscence	* Discontinue Bevacizumab
Hypertension	Patients should be monitored for the development or worsening of hypertension via frequent blood pressure measurement. Daily home monitoring is encouraged. Blood pressure measurements should occur

Event	Action to Be Taken
	after the patient has been in a resting position for ≥ 5 minutes. Repeat measurement of blood pressure for verification should be undertaken if the initial reading is ≥ 140 mmHg systolic and/or ≥ 90 mmHg diastolic blood pressure. • Grade 1 hypertension: Asymptomatic, transient (<24 hrs) increase by >20 mmHg (diastolic) or to >150/100 mmHg if previously within normal range. Intervention not indicated. • Grade 2 hypertension: Recurrent or persistent (>24 hr) or symptomatic increase by >20 mmHg (diastolic) or to >150/100 mmHg if previously within normal range. Monotherapy of anti-hypertensive may be indicated. Once controlled to <150/100 mmHg, patients may continue Bevacizumab therapy. • Grade 3 hypertension: Requiring more than one anti-hypertensive or more intensive therapy than previously. Bevacizumab should be withheld for persistent or symptomatic hypertension and should be permanently discontinued if BP is not controlled. • Grade 4 hypertension: Occurrence of grade 4 hypertension should lead to permanent discontinuation of Bevacizumab. All doses of anti-hypertensive medicines should be recorded at all visits.
Wound Healing Complications	Bevacizumab therapy should not be initiated for at least 28 days following major surgery or until the surgical wound is fully healed. In patients who experience wound healing complications during Bevacizumab treatment, Bevacizumab should be withheld until the wound is fully healed. Bevacizumab therapy should be withheld for elective surgery.

[00235] ***Concomitant Therapy***

[00236] All concurrent medical conditions and complications of the underlying malignancy are treated with discretion according to acceptable local standards of medical care. Patients receive analgesics, antiemetics, antibiotics, anti-pyretics, and blood products as necessary. Although warfarin-type anticoagulant therapies are permitted, careful monitoring of coagulation parameters is imperative, in order to avoid complications of any possible drug interactions. All concomitant medications, including transfusions of blood products, are recorded on the appropriate case report form. Dexamethasone and a 5-HT3 blocker (e.g., ondansetron or granisetron) are administered to all patients as premedication unless contraindicated for the individual patient. Antiemetics are prescribed as clinically indicated during the study period. Use of granulocyte colony-stimulating factors (G-CSF) is permitted to treat patients with neutropenia or neutropenic fever. Prophylactic use of G-CSF is permitted only in those patients who have had at least one episode of grade 3 or 4 neutropenia or neutropenic fever while receiving study therapy or have had documented grade 3 or 4 neutropenia or neutropenic fever while receiving prior anti-neoplastic therapy. Acute diarrhea and abdominal cramps, developing during or within 24 hours after MM-398 administration, can occur as part of a cholinergic syndrome. The syndrome is treated with atropine. Prophylactic or therapeutic administration of atropine is considered in patients experiencing cholinergic symptoms during the study unless clinically contraindicated.

[00237] Diarrhea can be debilitating and on rare occasions is potentially life-threatening. Guidelines developed by an ASCO panel for treating chemotherapy-induced diarrhea have been published (Benson AB, et al., (2004), J. Clin. Oncol. 2004; 22:2918-2926 and Wadler, S., et al. (1998), J. Clin. Oncol. 1998; 16(9):3169-3178). Suggested pharmacological approaches include: Oral loperamide administered as an initial 4-mg dose followed by 2-mg doses every 2 hours for irinotecan induced diarrhea; 2mg every 4 hours for 5-FU induced diarrhea. During the night, the patient can take 4mg every 4 hours for irinotecan induced diarrhea. Continue until diarrhea-free for ≥ 12 hours. This dose and regimen is moderately effective. Do not exceed 16mg per day.

[00238] If loperamide fails to control diarrhea within 24hours of onset then the use of octreotide is considered, administered at doses ranging from 100 micrograms twice daily to 500 micrograms three times daily, with a maximum tolerated dose of 2000 micrograms three times daily in a 5-day regimen.

[00239] Antibiotics such as cefpodoxim and cefixime are considered for patients who develop Grade 3 or 4 gastrointestinal (GI) toxicity following irinotecan+MM-398 therapy [42]. The antibiotic are started 5 days prior to the start of MM-398 therapy only if the patient experienced Grade 3 or 4 colitis, dehydration, diarrhea, abdominal pain, weight loss or vomiting during prior therapy with MM-398. If it is not feasible to start cefpodoxime or cefixime 5 days prior to therapy with MM-398, give at least 1 full day of cefpodoxime or cefixime prior to the start of MM-398 course. Patients are advised to drink water copiously throughout treatment (≥2l/day).

[00240] Surgical excision of the lesions is allowed under the following conditions:

[00241] Previous assessment of tumoral response at least after 4 cycles of treatment (2 months).

[00242] Intent to achieve a complete surgical resection (R0). After R0 or R1 secondary surgery (complete resection), patients will be removed from the study. In case of incomplete resection (R2), the patient remains in the study and should receive therapy according to treatment allocation.

[00243] ***Prohibited Therapy***

[00244] The following drugs are noted in the irinotecan prescribing information as interacting with irinotecan: St. John's Wort, CYP3A4-inducing anticonvulsants (e.g., phenytoin, phenobarbital, and carbamazepine), ketoconazole, itraconazole, troleandomycin, erythromycin, diltiazem and verapamil. Treatment with these agents and any other that interact with irinotecan, is avoided wherever possible.

[00245] MM-398

[00246] MM-398 is irinotecan (also known as CPT-11) encapsulated in a nanoliposomal drug delivery system in the form of the sucrose octasulfate salt of irinotecan. It is supplied as sterile, single-use vials containing 9.5 mL of MM-398 at a concentration of 5 mg/mL. The vials contain a 0.5 mL excess to facilitate the withdrawal of the label amount from each 10 mL vial.

[00247] The labels carry required regulatory instructions. The frequency at which the MM-398 is supplied to each individual center is adapted to the enrollment rate of the center and takes into consideration the expiry date of the MM-398. The MM-398 is shipped to the Hospital Pharmacist in accordance with local requirements. Upon receipt of MM-398, the Pharmacist inventories the MM-398 and completes the shipping form. MM-398 must be stored in a secured limited-access refrigeration at 2 to 8°C, with protection from light. Light protection is not required during infusion. MM-398 is not to be frozen. Responsible individuals should inspect vial contents for particulate matter before and after they withdraw the drug product from a vial into a syringe. The Hospital Pharmacist is responsible for the appropriate storage of the MM-398 at the study center. MM-398 must be diluted prior to administration. The diluted solution is physically and chemically stable for 6 hours at room temperature (15-30°C), but it is preferred to be stored at refrigerated temperatures (2-8°C), and protected from light. The diluted solution must not be frozen. Because of possible microbial contamination during dilution, it is advisable to use the diluted solution within 24 hours if refrigerated (2-8°C), and within 6 hours if kept at room temperature (15-30°C). MM-398 is administered by intravenous (IV) infusion over 90 minutes at a dose of 60-100 mg/m^2 every two weeks. The first Cycle Day 1 is a fixed day; subsequent doses are administered on the first day of each cycle, +/- 2 days. Prior to administration, the appropriate dose of MM-398 is diluted in 5% Dextrose Injection solution (D5W) to a final volume of about 250mL. Care is taken not to use in-line filters or any diluents other than D5W. MM-398 is not mixed with other drugs prior to infusion.

[00248] The actual dose of MM-398 to be administered is determined by calculating the patient's body surface area at the beginning of each cycle. A +/- 5% variance in the calculated total dose allows for ease of dose administration. MM-398 vials are single-use vials and unused portions of a vial are not stored for future use. The 90 minutes infusion period may be prolonged because of acute infusion-associated reactions or any other clinical needs. The infusion time can be reduced to 1 hour from cycle 2 if no acute infusion reaction has occurred in cycle 1. All patients are pre-medicated prior to MM-398 infusion with standard doses of dexamethasone and a 5-HT3 antagonist or other anti-emetic(s) according to standard institutional practices for irinotecan administration. Atropine may be prescribed prophylactically for patients who experienced acute cholinergic symptoms in previous cycles.

[00249] ***Free Irinotecan***

[00250] Irinotecan HCl is a well-known product, and available on the market as a sterile aqueous solution -- see irinotecan HCl (CAMPTOSAR®) U.S. Package Insert. Irinotecan is administered in accordance with standard procedures. For storage conditions one should follow the standard procedures for this compound. Other drugs should are not added to the infusion solution. Irinotecan is administered by intravenous (IV) infusion over 60 minutes at a dose of 90-150 mg/m^2 every two weeks. It is recommended that patients receive premedication with antiemetic agents per standard institutional practices. Prophylactic or therapeutic administration of atropine is considered in patients experiencing cholinergic symptoms.

[00251] *Drug Infusion Extravasation*

[00252] Should extravasation of the study drug at the infusion site occur, the steps below are to be followed: 1. IV is discontinued. 2. Infiltration is treated according to institutional guidelines for infiltration of a non-caustic agent.

[00253] *Study Assessments*

[00254] A medical history includes all pertinent prior medical conditions, surgeries or other medical procedures. Physical examination includes a careful assessment of all body systems, including the skin; central and peripheral nervous system; eyes, ears, nose and throat; respiratory and cardiovascular systems; abdomen and extremities. Particular attention is made to areas of possible neoplastic involvement. Vital signs include weight, resting blood pressure, pulse, respiratory rate and temperature. The Eastern cooperative oncology group (ECOG) Performance Score is obtained by questioning the patient about their functional capabilities. A 12 lead ECG includes a description of the cardiac rate, rhythm, interval durations and an overall impression. Tumor response is evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, to establish disease progression by computed tomography or MRI. In addition, other radiographic or scintigraphic procedures (such as radionuclide bone scans), are performed to assess sites of neoplastic involvement. The same method of assessment is used throughout the study. Investigators select target and non-target lesions in accordance with RECIST v1.1 guidelines (Eisenhauer, E. A., et al. (2009), EJC, 45(2), 228–47). Follow up measurements and response confirmation are in accordance with these guidelines. In the event the patient discontinues study treatment for reasons other than disease progression, a tumor assessment is completed as soon as possible relative to the date of study termination, unless performed within the prior 4 weeks, to ensure disease progression is not present and to assess overall disease status. In such patients, this assessment occurs no later than the date of the 30 day follow up visit and future assessments continue to take place every 8 weeks during the

follow-up period until objective disease progression or commencement of new anti-neoplastic therapy.

[00255] *Laboratory Procedures*

[00256] A complete blood count includes a white blood count (WBC) and differential, hemoglobin, hematocrit and platelet count. Serum chemistry includes electrolytes (sodium, potassium, chloride and bicarbonate), BUN, serum creatinine, glucose, bilirubin, AST, ALT, alkaline phosphatase, lactate dehydrogenase, uric acid, total protein, albumin, calcium, magnesium and phosphate. Whole blood and plasma are collected to potentially identify factors that may correlate with tumor response and resistance to MM-398. Examples of potential analyses include cytokine levels (e.g. MCSF1, and IL-6), growth factor markers (e.g. IGF1 and EGFR family receptors and ligands) and enzyme levels (e.g. MMP9). UGT1A family polymorphism may also be tested for either or both of UGT1A1*28 and UGT1A1*6 allele status. The tumor marker CEA is analyzed by the local lab. A coagulation profile includes a partial thromboplastin time and an international normalized ratio. A urinalysis includes descriptions of color and clarity; pH; specific gravity; and analyses of blood, glucose, ketones and total protein. A microscopic examination of the urine, to include WBC, RBC, bacteria and casts is performed if the urinalysis is abnormal. A urine or serum pregnancy test is obtained for all females of childbearing potential. Exempt female patients will include those who have undergone a bilateral oophorectomy or hysterectomy or who are menopausal (defined as absence of a menstrual cycle for at least 12 consecutive months). Plasma samples are collected during Cycle 1 to determine the levels of MM-398/irinotecan, SN-38 and SN-38G (SN-38 glucuronide, a less active metabolite of SN-38, levels of which may vary with UGT1A1 allele status). SN-38G/SN-38 concentration ratio may be calculated. This ratio may be useful to guide dose adjustments of irinotecan. The PK timepoints are outlined in the tables below. Optional timepoints can be considered. Additional analytes which may impact the pharmacokinetics of MM-398 are also measured from these samples for further analysis on clearance-related issues, if they occur, such as lipid-binding proteins.

[00257] **Table 4: Summary of PK Timepoints in Treatment and Follow-Up Phases**

Sample	Day	Hour	Time-point	Window
1	1	H0	Immediately prior to irinotecan infusion on Cycle 1Day 1	-5 mins
2	1	H+1	Immediately prior to the MM-398 infusion on Cycle 1Day 1	-5 mins
3	1	H+2.5	At the end of the MM-398 infusion	±5 mins
4	1	H+4.5	+2 hours after the completion of the MM-398 infusion	±30 mins
5	1	H+6.5	4hours after the completion of the MM-398 infusion	± 1 hour
6	2	H+26.5	24hours after the completion of the MM-398 infusion	±2hours
7	3		48hours after the completion of the MM-398 infusion, immediately prior to the Cycle 1 Day 3 post treatment biopsy	±1 hour
8	8		+168 hours/7 days after the completion of the Cycle 1 Day 1 MM-398 infusion	±24 hours
9	15		Immediately prior to MM-398 infusion on Cycle 2 Day 1	-24 hours

[00258] ***Tissue Collection***

[00259] A biopsy is performed (primary tumor or metastatic lesion), if clinically appropriate during the first treatment cycle (day 3), in order to isolate tumor tissue for further analysis of pharmacodynamics. A minimum of three passes is required to isolate tumor material for further analysis. If the tumor biopsy is not feasible during cycle 1, both plasma sample and tumor biopsy may be performed during cycle 2 (day 3). Archived FFPE tumor blocks or unstained paraffin slides containing tumor tissue, prepared at the time of initial diagnosis and at the time of metastasis (if available), are collected from each patient.

[00260] ***Image Data Collection***

[00261] CT imaging data is used for evaluation of RECIST v1.1 criteria. Volumetric analysis can be performed independently. Assessment of image density and CT heterogeneity or other advanced analysis strategies can be performed, as well.

[00262] ***Overall Survival/Post Study Follow-up***

[00263] Overall survival data is collected after a patient completes the 30 day follow-up visit, every 2 months (+/- 1 week) from the date of the 30 day follow-up visit. All patients are followed-up until death or study closure, whichever occurs first.

[00264] *Adverse Event Reporting*

[00265] *Adverse Event* An adverse event is any untoward medical occurrence in a patient administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign, including abnormal laboratory findings, symptoms, or diseases temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. Worsening of a medical condition for which the efficacy of the study drug is being evaluated is considered an adverse event.

[00266] *Unexpected Adverse Event* An unexpected adverse event is one for which the nature or severity of the event is not consistent with the applicable product information, e.g., the Investigator's Brochure.

[00267] *Serious Adverse Event* A serious adverse event (SAE) is any untoward medical occurrence that at any dose that: Results in death. Is life-threatening (an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe). Requires in-patient hospitalization or prolongation of existing hospitalization. Results in persistent or significant disability/incapacity. Is a congenital anomaly or birth defect. An SAE is also any other important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above. Examples of such events are intensive treatment in an emergency room for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse. The term "severe" is often used to describe the intensity (severity) of an event. The event itself may be of relatively minor medical significance (such as a severe headache). This is not the same as "serious", which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning.

[00268] *Documenting Adverse Events* Adverse event reporting begins on the date the patient provides informed consent to participate in the study. Information is elicited regarding the occurrence of adverse events through open-ended questioning of the patient, physical examination and review of laboratory results. All adverse events, whether serious or not, are recorded in the source documents and the adverse event page of the case report form (except as

noted below). All new events, as well as those that worsen in intensity or frequency relative to baseline, which occur after first administration of study drug through 30 days following the last dose of study drug, are recorded. Adverse events are followed through resolution, where possible. Adverse events that are ongoing at the time of treatment discontinuation are followed through the 30 day follow up assessment. However, new adverse events related to MM-398 and/or irinotecan must be reported any time the Investigator becomes aware of such an event, even if this occurrence is more than 30 days after the last dose of study drug. Laboratory, vital signs or ECG abnormalities are recorded as Adverse Events only if they are medically relevant: symptomatic, requiring corrective treatment, leading to discontinuation and/or fulfilling a seriousness criterion.

[00269] Information reported in the description of each adverse event includes:

[00270] • A medical diagnosis of the event (if a medical diagnosis cannot be determined, a description of each sign or symptom characterizing the event are recorded)

[00271] • The date of onset of the event

[00272] • The date of resolution of the event

[00273] • A determination of whether the event is serious or not

[00274] • Action taken: none; change in the study drug administration (e.g., temporary interruption in dosing); drug treatment required; non-drug treatment required; hospitalization or prolongation of hospitalization required (complete serious adverse event page); diagnostic procedure performed; patient discontinued from the study (complete Final Visit Section of the case report form)

[00275] • Outcome: resolved without sequelae; resolved with sequelae; event resolving; event ongoing; patient died (notify the Sponsor immediately, and complete the Serious Adverse Event page and the Final Visit section of the case report form)

[00276] *Reporting Serious Adverse Events* Any SAEs occurring during the screening period are reported only if they are believed to be related to a protocol procedure. All fatal or life-threatening adverse events or adverse event of special interest are reported. Within 24 hours of the event, the Serious Adverse Event Form is faxed to the appropriate contact whether full information regarding the event is known or not. Additional follow-up is required if complete information is not known. Source documentation of all examinations, diagnostic procedures, etc., which were completed with respect to the event are included with the SAE form.

[00277] In case of accidental or intentional overdose of any study drug, even if asymptomatic or not fulfilling a seriousness criterion, the overdose is to be reported immediately

(within 1 working day) using the AE and SAE forms. Overdose will be defined as ≥133% of planned dose.

[00278] *Determining the Severity and Relatedness of Adverse Events* Each adverse event is graded according to the NCI CTCAE Version 4.0(14 June 2010 v.4.03 reference), which may be found at http://evs.nci.nih.gov/ftp1/CTCAE/About.html. For events not listed in the CTCAE, severity is designated as mild, moderate, severe or life threatening or fatal which correspond to Grades 1, 2, 3, 4 and 5, respectively on the NCI CTCAE, with the following definitions: **Mild**: an event not resulting in disability or incapacity and which resolves without intervention;

[00279] **Moderate**: an event not resulting in disability or incapacity but which requires intervention; **Severe**: an event resulting in temporary disability or incapacity and which requires intervention; **Life-threatening**: an event in which the patient was at risk of death at the time of the event; **Fatal**: an event that results in the death of the patient.

[00280] The Investigator will attempt to determine if an adverse event is in some way related to the use of the study drug. This relationship is described as follows: **Unlikely**: The event is clearly due to causes distinct from the use of the study drug, such as a documented pre-existing condition, the effect of a concomitant medication a new condition which, based on the pathophysiology of the condition, and the pharmacology of the study drug, would be unlikely related to the use of the study drug; **Possible:** The event follows a reasonable temporal sequence from administration of the study drug and the event follows a known response pattern to the study drug BUT the event could have been produced by an intercurrent medical condition which, based on the pathophysiology of the condition, and the pharmacology of the study drug, would be unlikely related to the use of the study drug or the event could be the effect of a concomitant medication; **Probable:** The event follows a reasonable temporal sequence from administration of the study drug and the event follows a known response pattern to the study drug AND the event cannot have been reasonably explained by an intercurrent medical condition which or the event cannot be the effect of a concomitant medication; **Definite**: The event follows a reasonable temporal sequence from administration of the study drug, the event follows a known response pattern to the study drug and based on the known pharmacology of the study drug, the event is clearly related to the effect of the study drug; **Unknown**: Based on the evidence available, causality cannot be ascribed.

[00281] ***Statistical Considerations***

[00282] *General Statistical Considerations* The primary objective of this dose-escalation study is to assess the safety and tolerability of MM-398 in combination with free irinotecan and to determine the recommended dose (RD) for further studies sing this combination, when

administered in patients with unresectable advanced cancer. Descriptive statistics are utilized for safety, efficacy, and pharmacokinetic parameters. Categorical variables are summarized by frequency distributions (number and percentages of patients) and continuous variables are summarized by descriptive statistics (mean, standard deviation, median, minimum, maximum). All data are summarized by MM-398/irinotecan dose cohorts. The study population for safety and efficacy analyses is defined as all patients enrolled in the study who receive at least a partial infusion of MM-398. Patients who exit the study prior to receiving study medication or who withdraw in Cycle 1 for reasons unrelated to drug toxicity re replaced.

[00283] *Determination of Sample Size* Dose Escalation Phase

[00284] Approximately 5 dosing cohorts are evaluated. In this scenario, the minimum number of patients treated is 18 (5 cohorts x 3 + 3 patients) and the maximum number of patients treated is 30 (5 cohorts x 6 patients). The exact number of patients depends on the observed safety profile, which determines the number of patients per dose level, as well as the number of dose escalations required to meet MTD. Escalation to the next dose cohort depends on the background toxicity rate (i.e., probability of DLT at a given dose). The proposed plan for dose escalation provides at least 90% probability that dose escalation proceeds at doses associated with DLT probability of ≤10%. Table 9 shows the probability of escalation from cohort to cohort with various toxicity rates.

[00285] **Table 8: Probabilities of Dose Escalation**

	Background Toxicity Rate						
	1%	5%	10%	20%	30%	40%	50%
Initial cohort size is 3	0.999	0.973	0.906	0.709	0.494	0.309	0.172

[00286] *Treatment Assignment and Blinding* This is a dose-escalation, open-label study. Therefore, no randomization or blinding procedures is performed. Sequential cohorts of patients are treated at each dose with the dose escalation scheme described above.

[00287] *Statistical Analysis* Disposition of patients is presented by center and overall per MM-398 containing dose cohorts as patients are entered, treated, withdrawn and evaluable for efficacy and for safety. Demographic characteristics of patients are summarized by MM-398 dose cohort using descriptive statistics. The tumor response achieved is assessed per RECIST v1.1. The overall response rate (CR+PR), as well as the rates for the individual categories of response (i.e., CR, PR, SD, and PD), are estimated by the percent of patients achieving these criteria by dose cohort when possible and overall. The percent of patients who achieve clinical

benefit of response (CR + PR + SD), the median duration of response and the median progression free survival are displayed per dose cohort and overall. Response rate per treatment arm are presented with corresponding 95% CI calculated using the binomial distribution. Progression-free survival and overall survival are estimated using the Kaplan and Meier method.

[00288] *Analysis of Safety and Adverse Events* All patients who receive any amount of any of the study drugs are included in the final summaries and listings of safety data. All safety analyses are performed by dose level, treatment cycle and week, where appropriate. Summary tables present the number of patients observed with treatment-emergent adverse events and corresponding percentages by dose cohort. The denominator used to calculate incidence percentages consists of patients receiving at least one dose of MM-398 in that cohort. Within each summary table, the AEs are categorized by MedDRA body system and preferred term. Additional subcategories are based on event intensity (severity graded according to CTCAE v4.0) and relationship to study drug. Emphasis in the analysis will be placed on AEs classified as dose limiting. Deaths and SAEs are tabulated on a per-patient basis. Frequencies of adverse events are also summarized by body system and organs. Laboratory data is presented by dose cohort and visit. Abnormal laboratory values are assessed according to NCI CTCAE v4.0 where possible.

[00289] *Other analyses* Pharmacokinetic parameters are derived from blood PK samples and are analyzed using descriptive statistics, including the median, mean and 95% confidence intervals around parameter estimates by dose level. All PK parameters include C_{max}, T_{max}, AUC (area under the concentration curve), clearance, volume of distribution at steady state (Vd_{ss}), and the terminal elimination half-life. Estimation of the pharmacokinetic parameters is performed using standard non-compartmental methods. Spearman pairwise correlations are computed between the following measurements: Tumor associated macrophage levels, Tumor irinotecan levels, Tumor SN-38 and SN38G levels. Graphical and regression methods are used to explore potential relationships among correlated measurements. In addition, relationships between pharmacodynamics markers and efficacy response are evaluated in an exploratory manner. Analyses of pharmacodynamics markers include all patients with available data.

[00290] **Endnotes**

[00291] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure that come within known or customary practice within the art to which the invention pertains and may be

applied to the essential features set forth herein. The disclosure of each and every US, international, or other patent or patent application or publication referred to herein is hereby incorporated herein by reference in its entirety.

CLAIMS

What is claimed is:

1. A method of treating a cancer in a patient, the method comprising administering to the patient a therapeutically effective amount of an anti-cancer drug, wherein the therapeutically effective amount is administered by co-administration to the patient of a) a first amount of the drug in a sustained release injectable dosage form and b) a second amount of the drug in an immediate release injectable dosage form.

2. A drug in a sustained release injectable dosage form for use in a first amount for co-administration in combination with a second amount of the drug in an immediate release injectable dosage form, wherein the first amount combined with the second amount equals a therapeutically effective amount of the drug.

3. The method of claim 1, or the drug in a sustained release injectable dosage form of claim 2, wherein the co-administration yields an improved therapeutic index, improved pharmacokinetic profile, or therapeutic synergy, as compared to administration to matched patients of the first amount and the second amount as a combined amount, wherein the combined amount is in the sustained release injectable dosage form.

4. The method of claim 1, or the drug in a sustained release injectable dosage form of claim 2, wherein the sustained release and immediate release dosage forms are both comprised within a single formulation and are thereby injected together, and wherein the second amount is at least 10% of the first amount.

5. The method of claim 1, or the drug in a sustained release injectable dosage form of claim 2, wherein the sustained release and immediate release dosage forms of the drug are comprised within separate formulations and are mixed and injected together simultaneously, or are injected separately, said separate injections being performed simultaneously or sequentially.

6. The method of claim 5 wherein the sequential administrations do not overlap and thereby do not include a period of simultaneous administration.

7. A method of treating a cancer in a patient, the method comprising co-administering liposomal irinotecan and free irinotecan to the patient, wherein the co-administering is carried out in at least 1 cycle, wherein the cycle is a period of 2 weeks, and wherein for each cycle:
   (a) liposomal irinotecan is administered once at a dose of 60 or 80 mg/m^2; and
   (b) free irinotecan is administered once at a dose of 90 or 120 mg/m^2.

8. The method of claim, 7 wherein the cancer is an unresectable cancer.

9. The method of claim 7 or claim 8, wherein the cancer is colorectal cancer.

10. The method of any one of claims 7-9, further comprising co-administering leucovorin and 5-fluorouracil to the patient once in each cycle.

11. The method of claim 10, wherein for each cycle:
   (c) leucovorin is administered at a dose of 400 mg/m^2;
   (d) 5-fluorouracil is administered at a dose of 2400 mg/m^2.

12. The method of any one of claims 7-11, further comprising administering bevacizumab to the patient.

13. The method of claim 12, wherein the bevacizumab is administered once per cycle by infusion at a dose of 5 mg/kg or 10 mg/kg.

14. The method of any one of claims 7-13, wherein the cancer is metastatic colorectal cancer.

15. The method of any one of claims 12-14, wherein bevacizumab is administered intravenously over 30-90 minutes.

16. The method of any one of claims 10-15, wherein 5-fluorouracil is administered intravenously over 46 hours.

17. The method of any one of claims 10-16, wherein leucovorin is administered intravenously over 2 hours.

18. A method of treating unresectable advanced colorectal cancer in a patient, the method comprising co-administering each of liposomal irinotecan and free irinotecan to the patient, wherein the method comprises at least 1 cycle, wherein the cycle is a period of 2 weeks, and wherein once for each cycle:

(a) the liposomal irinotecan is administered at a dose of 60 or 80 mg/m^2;

(b) the free irinotecan is administered at a dose of 90 or 120 mg/m^2.

19. The method of claim 18, wherein the method further comprises co-administering each of leucovorin and 5-fluorouracil to the patient, wherein once for each cycle:

(c) leucovorin is administered at a dose of 400 mg/m^2;

(d) 5-fluorouracil is administered at a dose of 2400 mg/m^2.

20. The method of claim 19, wherein the method further comprises co-administering bevacizumab to the patient, wherein once for each cycle:

(e) bevacizumab is administered at a dose of 5 mg/kg.

21. The method of any one of the preceding claims, wherein the liposomal irinotecan is administered intravenously over 90 minutes and the free irinotecan is administered intravenously over 60 minutes, optionally wherein, in each cycle, the free irinotecan is administered prior to the liposomal irinotecan.

22. The method of any one of claims 7-21, wherein, in each cycle, the free irinotecan and the liposomal irinotecan are administered prior to the 5-fluorouracil.

23. The method of any one of claims 7-22, wherein, in each cycle, the leucovorin is administered prior to administration of the 5-fluorouracil.

24. The method of any one of claims 7-23, wherein, in each cycle, the liposomal irinotecan and the free irinotecan are administered prior to the leucovorin, and the 5-fluorouracil.

25. The method of any one of the preceding claims, wherein, in each cycle, the liposomal irinotecan is irinotecan sucrosofate liposome injection (irinotecan liposome injection MM-398).

26. A formulation of liposomal irinotecan for co-administration with free irinotecan, bevacizumab, leucovorin, and 5-fluorouracil in at least one cycle, wherein the cycle is a period of 2 weeks, and wherein, once in each cycle:
    (a) liposomal irinotecan is administered at a dose of 60 or 80 mg/m^2;
    (b) free irinotecan is administered at a dose of 90 or 120 mg/m^2;
    (c) leucovorin is administered at a dose of 400 mg/m^2;
    (d) 5-fluorouracil is administered at a dose of 2400 mg/m^2; and
    (e) bevacizumab is administered at a dose of 5 mg/kg.

27. A formulation of liposomal irinotecan for co-administration with free irinotecan, in at least one cycle, wherein the cycle is a period of 2 weeks, and wherein, once in each cycle:
    (a) liposomal irinotecan is administered at a dose of 60 or 80 mg/m^2;
    (b) free irinotecan is administered at a dose of 90 or 120 mg/m^2.

28. The formulation of claim 19 or 20, wherein the liposomal irinotecan is administered intravenously over 60 minutes or 90 minutes.

29. The formulation of any one of claims 26-28, wherein the free irinotecan is administered intravenously over 60 minutes.

30. The formulation of any one of claims 26 or 28-30, wherein the bevacizumab is administered intravenously over 30-90 minutes.

31. The formulation of any one of claims 26 or 28-30, wherein the 5-fluorouracil is administered intravenously over 46 hours.

32. The formulation of any one of claims 26 or 28-31, wherein the leucovorin is administered intravenously over 2 hours.

33. The formulation of any one of claims 26-31, wherein the liposomal formulation of irinotecan is irinotecan sucrosofate liposome injection (MM-398).

34. A kit for treating a cancer in a human patient, the kit comprising a first container holding a second container and a third container and (optionally) instructions, the second container

comprising a dose of liposomal irinotecan the third container comprising a dose of free irinotecan, and the instructions calling for co-administering the liposomal irinotecan and the free irinotecan to a cancer patient.

35. The method, drug or formulation of any of claims 1-2 or 4-34, wherein the co-administration results in an improved therapeutic index, or improved pharmacokinetics, or in therapeutic synergy.

36. A method of improving the pharmacokinetic profile of a drug treatment, the method comprising administering the drug enterally or parenterally in doses separated by an interval of a day or more, or a week, or two weeks, or three weeks, or a month or more, in unencapsulated form, and co-administering the same drug parenterally at the same interval in a liposomally encapsulated injectable form, such that the pharmacokinetic profile is improved in that the drug reaches therapeutic levels at a site of action faster than if administered only in the encapsulated form, and maintains therapeutic levels at the site of action longer than if administered at the same intervals only in the unencapsulated form.

37. The method of claim 36, wherein the unencapsulated drug and the liposomally encapsulated drug are administered sequentially or simultaneously.

38. The method of claim 37, wherein the drug is an anti-cancer drug selected from a topoisomerase I inhibitor, a topoisomerase II inhibitor, a taxane, a vinca alkaloid, a platinum salt, an alkylating agent, or an anti-metabolite.

39. The method of claim 38 wherein the topoisomerase I inhibitor is irinotecan, SN-38 or topotecan, the topoisomerase II inhibitor is an anthracycline, teniposide, or etoposide, the taxane is paclitaxel, docetaxel, cabazitaxel, or tesetaxel, the vinca alkaloid is vincristine, vinblastine, or vinorelbine, the platinum salt is oxaliplatin, cisplatin or carboplatin, the alkylating agent is bendamustine, busulfan, carmustine, chlormbucil, cyclophosphamide, ifosfamide, lomustine, mechlorethamine, melphalan, streptazocin, thiotepa, or uramustine, and the anti-metabolite is 5-FU, capecitabine, cytarabine, gemcitabine, methotrexate, pemetrexed, or tegafur .

40. The method of claim 39, wherein the anthracycline is doxorubicin, daunorubicin, bleomycin, dactinomycin, epirubicin, idarubicin, mitomycin, or mitoxantrone.

41. The method of any one of claims 36-40, wherein the interval is two weeks.

42. The method of any one of claims 36-41, wherein the co-administration of the drug in unencapsulated form and of the same drug in liposomally encapsulated form produces an improved therapeutic index, or improved pharmacokinetics, or therapeutic synergy.

43. A method of treating a cancer in a patient, the method comprising at least one cycle of co-administration of 1) a chemotherapeutic agent that is a sustained release dosage form and 2) of the same chemotherapeutic agent that is an immediate release dosage form, wherein the co-administration of both dosage forms provides an improved therapeutic index, or improved pharmacokinetics, or therapeutic synergy.

44. The method of claim 43 wherein the two dosage forms are both comprised within a single formulation and are thereby injected together.

45. The method of claim 43 wherein the two dosage forms of the chemotherapeutic agent are comprised within separate formulations and are mixed and injected together simultaneously or are injected separately, said separate injections being performed simultaneously or sequentially.

46. The method of claim 45 wherein the sequential administrations do not overlap and thereby do not include a period of simultaneous administration.

47. The method of any one of claims 39-46 wherein the chemotherapeutic agent is an alkylating agent, an anti-metabolite, an anti-microtubule agent, a topoisomerase inhibitor or a cytotoxic antibiotic.

48. The method of claim 47 wherein the alkylating agent is a nitrogen mustard, a nitrosourea, a tetrazine, an aziridine, an organoplatinum agent, procarbazine or hexamethylmelamine.

49. The method of claim 47 wherein the anti-metabolite is an anti-folate, a fluoropyrimidine, a deoxynucleoside analogue, or a thiopurine.

50. The method of claim 47 wherein the anti-microtubule agent is a taxane, a vinca alkaloid or vinca alkaloid derivative.

51. The method of claim 47 wherein the topoisomerase inhibitor is a camptothecin topoisomerase I inhibitor or a topoisomerase II inhibitor.

52. The method of claim 54 wherein the camptothecin topoisomerase I inhibitor is topotecan or irinotecan.

53. The method of claim 52 wherein the camptothecin topoisomerase I inhibitor is irinotecan and the sustained release dosage form is in a liposomal, hyaluronate, or PEGylated form and the immediate release dosage form is in the form of an irinotecan hydrochloride solution or its analogs or derivatives.

54. The method of claim 53 wherein the sustained release dosage form is liposomal irinotecan sucrosofate and, for each cycle of co-administration:

(a) the liposomal irinotecan sucrosofate is administered at a dose range between 60 and 100 mg/m^2; and

(b) the irinotecan hydrochloride is administered at a dose range between 90 and 180 mg/m^2.

55. The method of claim 54 comprising at least a second cycle, wherein the cycles are at two week intervals.

56. The method of claim 54, further comprising co-administration of an effective amount of each of leucovorin (folinic acid) and 5-flurouracil.

57. The method of claim 47, further comprising co-administration of an effective amount of each of leucovorin and 5-flurouracil.

58. The method of claim 57, wherein, in each cycle of co-administration, the leucovorin is administered at a dose of 400 mg/m^2 and the 5-fluorouracil is administered at a dose of 2400 mg/m^2.

59. The method of any one of claims 36-58, the method further comprising co-administering an effective amount of an anti-angiogenic agent to the patient.

60. The method of claim 59 wherein the anti-angiogenic agent is bevacizumab.

61. The method of claim 60 wherein the bevacizumab is co-administered at a dose of 5 mg/kg Q2W or 10 mg/kg Q2w or 15 mg/kg Q3W.

62. The method of claim 38, wherein the topoisomerase I inhibitor is irinotecan and the liposomally encapsulated irinotecan is irinotecan sucrosofate liposome injection (MM-398).

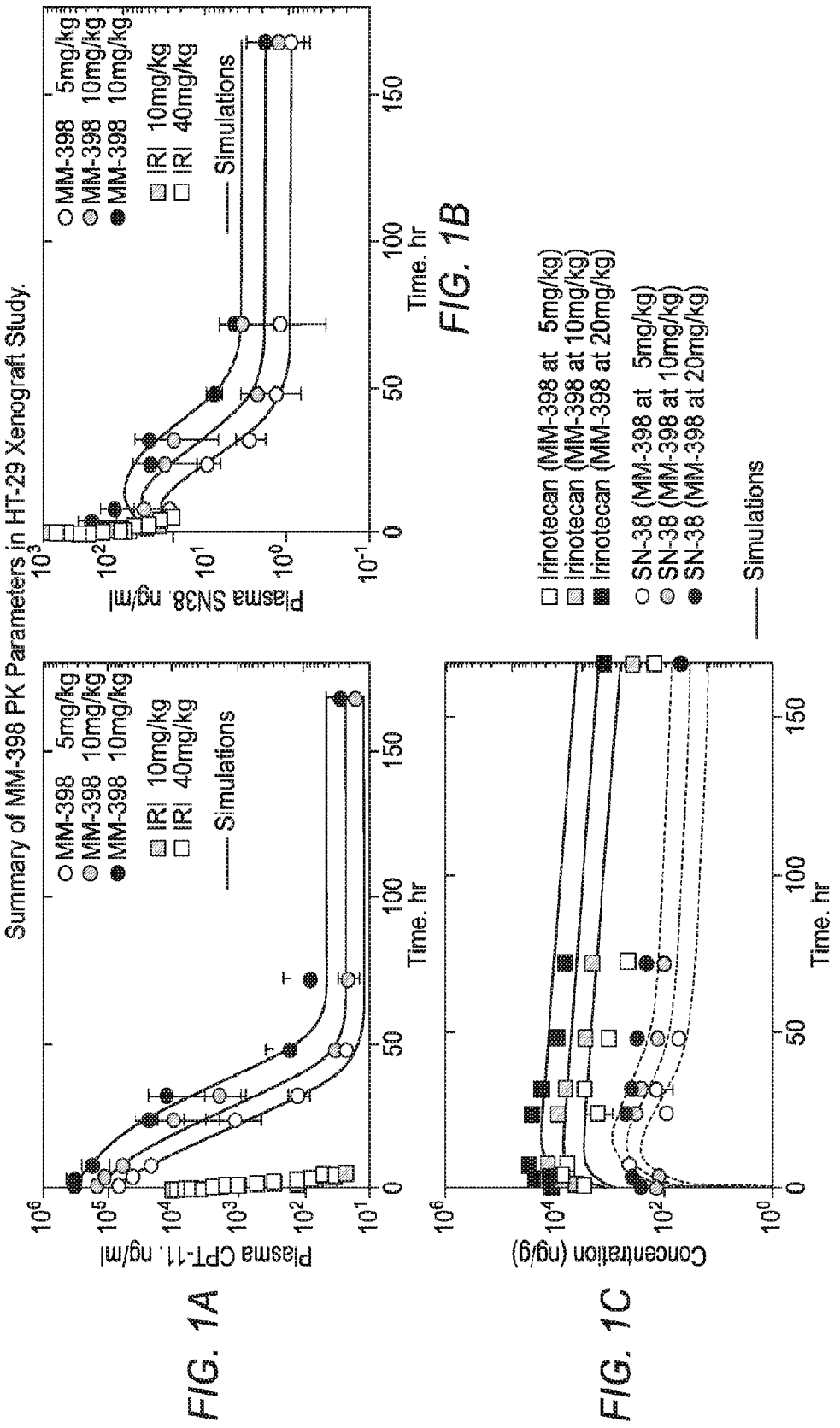

FIG. 1A

FIG. 1B

FIG. 1C

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/027515

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/00 A61K39/395 A61K31/4745 A61K31/513 A61K31/519
A61K45/06 A61P35/00 A61P35/02
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, CHEM ABS Data, 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.
X,P	BENOIST CHIBAUDEL ET AL: "PEPCOL: a GERCOR randomized phase II study of nanoliposomal irinotecan PEP02 (MM-398) or irinotecan with leucovorin/5-fluorouracil as second-line therapy in metastatic colorectal cancer", CANCER MEDICINE, vol. 5, no. 4, 24 January 2016 (2016-01-24), pages 676-683, XP055280612, GB ISSN: 2045-7634, DOI: 10.1002/cam4.635 the whole document ----- -/--	1-62

[X] Further documents are listed in the continuation of Box C.

[X] 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

"E" earlier application or patent but published on or after the international filing date

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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	Date of mailing of the international search report
20 June 2016	27/06/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Taylor, Mark

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. C. BENDELL ET AL: "Treatment Patterns and Clinical Outcomes in Patients With Metastatic Colorectal Cancer Initially Treated with FOLFOX-Bevacizumab or FOLFIRI-Bevacizumab: Results From ARIES, a Bevacizumab Observational Cohort Study", THE ONCOLOGIST, vol. 17, no. 12, 26 September 2012 (2012-09-26), pages 1486-1495, XP055280593, US ISSN: 1083-7159, DOI: 10.1634/theoncologist.2012-0190 the whole document --- XIJIAN ZHOU ET AL: "Clinical Analysis of Bevacizumab Plus FOLFIRI Regimen as Front-Line Therapy for Chinese Patients with Advanced Colorectal Cancer", JOURNAL OF CANCER THERAPY, vol. 02, no. 04, 1 January 2011 (2011-01-01), pages 470-474, XP055280602, ISSN: 2151-1934, DOI: 10.4236/jct.2011.24063 the whole document --- WO 2013/188586 A1 (MERRIMACK PHARMACEUTICALS INC [US]) 19 December 2013 (2013-12-19) abstract page 3, line 4 - page 6, line 14 page 8, line 23 - page 9, line 21 page 11, line 18 - page 12, line 5 page 12, line 6 - line 14 page 13, line 1 - page 14, line 10 examples 1-7 claims 1-27 --- WO 2004/093795 A2 (CELATOR TECHNOLOGIES INC [CA]; TARDI PAUL [CA]; HARASYM TROY [CA]; WEB) 4 November 2004 (2004-11-04) abstract paragraph [0002] paragraph [0008] examples 1-27 claims 1-24 ---	1-62 1-62 1-62 1-62

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/027515

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(19) World Intellectual Property Organization
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(54) Title: STABILIZING CAMPTOTHECIN PHARMACEUTICAL COMPOSITIONS

(57) Abstract: Irinotecan phospholipid liposomes with improved storage stability are provided, with related methods of treatment and manufacture. The irinotecan liposomes can have reduced formation of lyso-phosphatidylcholine (lyso-PC) during storage, and prior to administration to a patient.

STABILIZING CAMPTOTHECIN PHARMACEUTICAL COMPOSITIONS

PRIORITY CLAIM

[0001] This patent application claims the benefit of U.S. Provisional Patent Application Serial Nos. 62/242,835 (filed October 16, 2015), 62/242,873 (filed October 16, 2015), 62/244,061 (filed October 20, 2015), and 62/244,082 (filed October 20, 2015), each of which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] This disclosure relates to stabilizing pharmaceutical compositions comprising camptothecin compounds, including liposomal camptothecin pharmaceutical formulations stabilized to reduce formation of lyso-lipid formation during storage.

BACKGROUND

[0003] Camptothecin compounds (such as irinotecan or topotecan) can be used to treat a tumor and/or cancer within the human body. For example, injectable liposome pharmaceutical products for the treatment of certain forms of cancer can be prepared as dispersions of liposomes encapsulating camptothecin compounds. The liposomal camptothecin compositions can encapsulate the camptothecin compound together with a polyanionic trapping agent within a liposome comprising cholesterol and one or more phospholipid(s) ("PL"). However, the hydrolysis of phospholipids and the hydrolysis of the active lactone structure in camptothecin can occur in camptothecin liposomes having one or more phospholipids. The hydrolytic decomposition of a liposomal phospholipid such as a phosphatidylcholine ("PC"+) can alter the release of the camptothecin compound, e.g., irinotecan, from the liposomes. The first step in the hydrolysis of PL (such as PC) can lead to the formation of lyso-PL (such as lysophosphatidylcholine ("lyso-PC"), which is a glycerylphosphocholine fatty acid monoester).

[0004] Liposomal camptothicin compositions are affected by pH in at least two respects. First, the hydrolytic decomposition of liposomal captothecin (e.g., liposomal irinotecan) phospholipids tends to be pH dependent, with a pH of 6.0 or 6.5 believed to minimize hydrolysis of phosphatidylcholine. Conditions where the pH is above 6.5 tend to increase (1) the conversion of camptothecin compounds, e.g. irinotecan, to the less active carboxylate form and (2) the amount of lyso-PC in liposomes. Second, camptothecin compounds undergo a pH-dependent conversion between a less active carboxylate form (predominating at neutral

and alkaline pH) and a more active lactone form predominating at acidic pH. For example, the conversion of the carboxylate form of irinotecan to the lactone form occurs primarily between pH 6.0 (about 85% of the irinotecan is in the more active lactone form) and pH 7.5 (only about 15% of irinotecan is in the more active lactone form). At pH 6.5, about 65% of irinotecan is in the more active lactone form.

[0005] The stability of phospholipid-containing liposomal camptothecins prepared at a pH of 6.5 was unexpectedly found to be adversely affected by the formation of lyso-PC during storage under refrigerated conditions (2-8 °C). For example, an irinotecan liposome composition of Sample 12 (irinotecan sucrose octasulfate encapsulated in irinotecan liposomes comprising DSPC, cholesterol and MPEG-2000-DSPE in a 3:2:0.015 mole ratio, prepared at pH 6.5) subsequently generated levels of lyso-PC in excess of 30 mol% (with respect to the total amount of phosphatidylcholine in the irinotecan liposome compositions) during the first 3 months after manufacture (and over 35mol% lyso-PC generated during the first 9 months) of refrigerated storage (2-8°C).

[0006] Therefore, there remains a need for stabilized camptothecin pharmaceutical compositions. For example, there is a need for more stable, improved liposomal formulations of irinotecan generating less lyso-PC during refrigerated storage at 2-8 °C after manufacturing. The present invention addresses this need.

SUMMARY

[0007] The present invention provides novel camptothecin pharmaceutical compositions (e.g., liposomal irinotecan) with improved stability, including camptothecin liposomal compositions comprising ester-containing phospholipids with reduced rates of formation of lyso-phospholipid ("lyso-PL") (e.g., lyso-phosphatidylcholine, or "lyso-PC"). The present invention is, in part, based on the surprising recognition that liposomal compositions of camptothecin compounds (e.g., irinotecan) can be manufactured that generate reduced amounts of lyso-phospholipids after extended storage at 2-8 °C. The manufacture of such stabilized liposomal compositions is made possible by the unexpected finding that controlling specific parameters during liposome manufacture (the ratio of drug-to-phospholipid relative to the amount of trapping agent, the pH of the liposomal preparation and the amount of trapping agent counter-ion in the liposomal preparation) synergistically reduces the formation of lyso-phospholipids during storage of the camptothecin liposomal preparation. The invention provides extremely valuable information for designing and identifying improved

liposome compositions, which are more robust, while reducing costs associated with the development of such compositions.

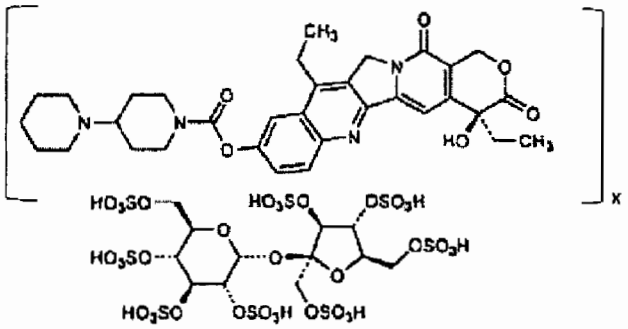
[0008] Stabilized camptothecin compositions comprising one or more phospholipid(s) (including PEG-containing phospholipid(s)), preferably form not more than 20 mol% of lyso-PL (relative to the total liposome phospholipids) during storage for the first 6 months of storage at 4 °C and/or not more than 25 mol% of lyso-PL during storage for the first 9 months of storage at 4 °C. The stabilized irinotecan liposomes preferably form lyso-PL at an average rate of less than about 2 mol% (e.g., 0.5-1.5 mol%) lyso-PL per month during the first 9 months of storage at 4 °C, following manufacture of the camptothecin compositions. Preferred stabilized camptothecin compositions include irinotecan or a salt thereof (e.g. irinotecan sucrose octasulfate) in liposomal irinotecan compositions comprising cholesterol and one or more phospholipid(s) (including PEG-containing phospholipid(s)), that form not more than 20 mol% of lyso-PC (relative to the total liposome phospholipids) during storage for 6 months at 4 °C and/or not more than 25 mol% of lyso-PC during storage for 9 months at 4 °C (e.g., during the first 6 and/or 9 months of stability testing after manufacturing). The stabilized irinotecan liposomes can form lyso-PC at a rate of less than about 2 mol% (e.g., 0.5-1.5 mol%) lyso-PC per month during storage at 4 °C (e.g., during the first 9 months of stability testing after manufacturing). Stabilized phosphatidylcholine-containing irinotecan liposome compositions can generate less than 1 mg lyso-PC during the first 9 months of stability testing at 2-8 °C after manufacturing.

[0009] In a first embodiment, stabilized liposomal camptothecin compositions have a pH greater than 6.5 (e.g., 7.0-7.5, including 7.25, 7.3 or 7.5) and comprise liposomes encapsulating irinotecan and a sulfate polyanionic trapping agent (e.g, irinotecan sucrosofate, or "SOS") having an irinotecan/sulfate compound gram-equivalent ratio ("ER") that is greater than 0.9 (e.g., 0.9-1.1). The ER can be calculated for an irinotecan SOS liposome preparation by determining molar amounts of liposomally co-encapsulated irinotecan (I) and sulfate compound (S) per unit (e.g., 1 mL) of the liposome composition, and using the formula: ER = I/(SN), where N is valency of the sulfate compound anion (e.g., for sucrosofate N is 8, and for free sulfate, SO_4^{2-}, N is 2). Preferably, the sulfate compound (S) is sucrose octasulfate, containing 8 sulfate moieties per mol of SOS.

[0010] In a second embodiment, stabilized liposomal camptothecin compositions are obtained using particular ratios of the camptothecin, an anionic trapping agent and liposome-forming phospholipids having a Stability Ratio ("SR") that is preferably greater than about 950 (e.g., 950-1050), including irinotecan liposomes prepared with a SR of greater than about

990 (e.g., 990-1,100, including 992-1,087). This embodiment provides manufacturing criteria that are predicative of liposome stability as reflected by a Stability Ratio, as more fully explained below. This embodiment of the invention is based in part on the discovery that when phospholipid-based camptothecin-containing liposomes are made by reacting (1) camptothecin compound(s) (e.g., irinotecan, topotecan, and the like) with (2) liposomes encapsulating a polysulfated anionic trapping-agent (e.g., sucrose octasulfate), the stability of the resulting drug-loaded liposomes depends on initial concentration of sulfate groups in the trapping-agent-liposomes and the ratio of camptothecin encapsulated to phospholipid in the liposomes. The Stability Ratio, is defined as follows: SR = A/B, where: A is the amount of irinotecan moiety encapsulated in trapping agent liposomes during the drug loading process, in grams equivalent to the irinotecan free anhydrous base, per mole of phospholipid in the composition; and B is the concentration of sulfate groups in the sucrosofate (or other trapping agent) solution used to make the trapping agent liposomes, expressed in mole/L (based on the concentration of sulfate groups). The Stability Ratio surprisingly predicted dramatic reductions in the formation of lyso-PC in phospholipid-based camptothecin-containing liposomes, even at pH 6.5; phosphatidylcholine-containing irinotecan liposomes prepared with a Stability Ratio of about 942 (Sample 3) generated about 36 mol% lyso-PC, compared to about 24 mol% lyso-PC generated in irinotecan liposomes prepared with a Stability Ratio of about 990 (Sample 2), after 9 months of storage at 4 °C (i.e., increasing the Stability Ratio by about 5% resulted in a 34% reduction in lyso-PC generation under these conditions). In contrast, increasing the Stability Ratio of irinotecan liposomes by about 30% from 724 (Sample 12) to 942 (Sample 3) resulted in about 1% more lyso-PC generated after 9 months of storage at 4 °C (e.g., compare 35.7 mol% lyso-PC in Sample 3 to 35.4 mol% lyso-PC in Sample 12).

[0011] In a third embodiment, novel stabilized compositions of liposomes encapsulating irinotecan having reduced amounts of lyso-phosphatidylcholine (lyso-PC) generated during storage at 2-8 °C can comprise the irinotecan composition of formula (I), where x is 8.



(I).

The liposomal irinotecan can comprise the composition of formula (I) encapsulated in liposomes. Preferably, the composition of formula (I) is formed (e.g., precipitated) within liposomes comprising cholesterol and one or more phospholipid(s) (e.g., including PEG-containing phospholipid(s)). For example, the compound of formula (I) can be formed within the liposomes by reacting (1) a camptothecin compound(s) (e.g., irinotecan, topotecan, and the like) with (2) liposomes encapsulating a polysulfated anionic trapping-agent (e.g., sucrose octasulfate), in a process that forms a stabilized liposomal irinotecan composition. Preferably, the liposomal irinotecan composition has a pH greater than 6.5 (e.g., 7.0-7.5, including 7.25, 7.3 and 7.5).

[0012] Preferred stabilized camptothecin compositions include liposomal irinotecan compositions comprising irinotecan or a salt thereof (e.g. irinotecan sucrose octasulfate) encapsulated within irinotecan liposomes comprising cholesterol and the phospholipids 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and methoxy-terminated polyethylene glycol-distearoylphosphatidyl ethanolamine (e.g., MPEG-2000-DSPE) in an aqueous isotonic buffer, said liposomal irinotecan composition containing (or forming) less than 10 mol% lyso-phosphatidylcholine (lyso-PC) after the first 3 months of storage at 2-8 °C, containing (or forming) less than 20 mol% lyso-phosphatidylcholine (lyso-PC) after the first 6 months (or 180 days) of storage at 2-8 °C, and/or containing (or forming) less than 25 mol% lyso-phosphatidylcholine (lyso-PC) after the first 9 months of storage at 2-8 °C (e.g., during the first 9 months of stability testing after manufacturing).

[0013] The irinotecan liposomes preferably comprise cholesterol, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and methoxy-terminated polyethylene glycol-distearoylphosphatidyl ethanolamine (e.g., MPEG-2000-DSPE) in a 3:2:0.015 mole ratio, encapsulating 500 mg (±10%) irinotecan per mmol total liposome phospholipid. Stabilized liposomal irinotecan compositions preferably comprise irinotecan liposomes providing a total

of about 4.3 mg irinotecan moiety per mL of the liposomal irinotecan composition, with at least about 98% of the irinotecan encapsulated in the irinotecan liposomes (e.g., as irinotecan sucrose octasulfate, such as a compound of Formula (I) above). Certain preferred liposomal composition are storage stabilized liposomal irinotecan compositions having a pH of 7.00-7.50 (e.g., 7.0, 7.25, 7.3, 7.5) and comprising a dispersion of irinotecan liposomes encapsulating irinotecan sucrose octasulfate in unilamellar bilayer vesicles consisting of cholesterol and the phospholipids 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE), at a concentration of irinotecan moiety equivalent to, in g of irinotecan free anhydrous base, 500 mg (±10%) irinotecan per mmol total liposome phospholipid and 4.3 mg irinotecan per mL of the liposomal irinotecan composition, the storage stabilized liposomal irinotecan composition stabilized to form less than 1 mg/mL Lyso-PC during the first 6 months of storage at 4 °C. For example, certain preferred pharmaceutical liposomal irinotecan compositions comprise irinotecan or a salt thereof (e.g. irinotecan sucrose octasulfate) encapsulated in irinotecan at 4.3 mg/mL irinotecan moiety, 6.81 mg/mL of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 2.22 mg/mL cholesterol, and 0.12 mg/mL methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE) in an aqueous isotonic buffer, said liposome composition containing less than 10 mol% lyso-phosphatidylcholine (lyso-PC) after 3 months of storage at 2-8 °C, containing less than 20 mol% lyso-phosphatidylcholine (lyso-PC) after 6 months (or 180 days) of storage at 2-8 °C, and/or containing less than 25 mol% lyso-phosphatidylcholine (lyso-PC) after 9 months of storage at 2-8 °C.

[0014] In some embodiments, the liposomal composition is made by a method comprising contacting a solution containing irinotecan moiety with a trapping agent liposome encapsulating a triethylammonium (TEA), and sucrose octasulfate (SOS) trapping agent at a concentration of 0.4-0.5 M (based on the sulfate group concentration), as TEA_8SOS (preferably the TEA_8SOS trapping agent solution)under conditions effective to load 500 g (±10%) of the irinotecan moiety / mol phospholipid into the trapping agent liposome containing PL and permit the release of the TEA cation from the trapping agent liposome, to form the irinotecan SOS liposomes, and (b) combining the irinotecan SOS liposomes with 2-[4-(2-hydroxyethyl) piperazin-1-yl]ethanesulfonic acid (HEPES) to obtain an irinotecan liposome composition having a pH of 7.25-7.50, to obtain an irinotecan liposome composition stabilized to form less than 10 mol% lyso-phosphatidylcholine (Lyso-PC) (with

respect to the total amount of phosphatidylcholine in the irinotecan liposome composition) during 3 months of storage at 4 °C.

[0015] For instance, the invention provides an irinotecan liposome composition comprising stabilized irinotecan liposomes encapsulating irinotecan sucrose octasulfate (SOS) in an unilamellar lipid bilayer vesicle approximately 110 nm in diameter consisting of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE), wherein the stabilized irinotecan liposomes are obtained by a process comprising the steps of: (a) contacting irinotecan with a trapping agent liposome encapsulating a triethylammonium (TEA) cation, and sucrose octasulfate (SOS) trapping agent at a concentration of 0.4-0.5 M (based on the sulfate group concentration), as TEA_8SOS under conditions effective to load 500 g (±10%) of the irinotecan moiety / mol phospholipid into the trapping agent liposome and permit the release of the TEA cation from the trapping agent liposome, to form the irinotecan SOS liposomes, and (b) combining the irinotecan SOS liposomes with 2-[4-(2-hydroxyethyl) piperazin-1-yl]ethanesulfonic acid (HEPES) to obtain an irinotecan liposome composition having a pH of 7.25-7.50, to obtain an irinotecan liposome composition stabilized to form less than 10 mol% lyso-phosphatidylcholine (Lyso-PC) (with respect to the total amount of phosphatidylcholine in the irinotecan liposome compositions) during 3 months of storage at 4 °C.

[0016] The liposomal irinotecan compositions are useful in the treatment of patients diagnosed with various forms of cancer. For example, liposomal irinotecan can be administered for the treatment of small cell lung cancer (SCLC) without other antineoplastic agents. In some embodiments, the liposomal irinotecan compositions are administered in combination with other antineoplastic agents. For example, a liposomal irinotecan composition, 5-fluorouracil, and leucovorin (without other antineoplastic agents) can be administered for treatment of patients diagnosed with metastatic adenocarcinoma of the pancreas with disease progression following gemcitabine-based therapy. A liposomal irinotecan composition, 5-fluorouracil, leucovorin, and oxaliplatin (without other antineoplastic agents) can be administered for treatment of patients diagnosed with previously untreated pancreatic cancer. A liposomal irinotecan composition, 5-fluorouracil, leucovorin, and an EGFR inhibitor (e.g., an oligoclonal antibody EGFR inhibitor such as MM-151) can be administered for treatment of patients diagnosed with colorectal cancer.

[0017] Unless otherwise stated in this specification, liposomal compositions contain an amount of irinotecan in grams (in free base or salt form) to moles of phospholipid in a ratio

equivalent to that provided by either 471 g or 500 g (±10%) irinotecan free base per mol phospholipid.

[0018] As used herein (and unless otherwise specified), "irinotecan moiety" refers solely to the irinotecan lactone; i.e., the irinotecan lactone free base, anhydrous.

[0019] As used herein (and unless otherwise specified), the term "camptothecin" includes camptothecin and camptothecin derivatives including irinotecan, topotecan, lurtotecan, silatecan, etirinotecan pegol, TAS 103, 9-aminocamptothecin, 7-ethylcamptothecin, 10-hydroxycamptothecin, 9-nitrocamptothecin, 10,11-methylenedioxycamptothecin, 9-amino-10,11-methylenedioxycamptothecin, 9-chloro-10,11-methylenedioxycamptothecin, (7-(4-methylpiperazinomethylene)-10,11-ethylenedioxy-20(S)-camptothecin, 7-(4-methylpiperazinomethylene)-10,11-methylenedioxy-20(S)-camptothecin, and 7-(2-N-isopropylamino)ethyl)-(20S)-camptothecin, and stereoisomers, salts and esters thereof.

[0020] As used herein (and unless otherwise specified), "DLS" refers to dynamic light scattering and "BDP" refers to bulk drug product.

[0021] In some embodiments, the liposomes of the present invention encapsulate one or more agents that trap the pharmaceutical drug within liposomes (hereafter referred to as trapping agents).

[0022] As used in this specification, "extended release compositions" include irinotecan compositions that afford 80 to 125% of the following pharmacokinetic parameters when administered to humans at a dose corresponding to 70 mg/m^2 of irinotecan free base once every two weeks: Cmax 37.2 (8.8) μg irinotecan (as free base anhydrous)/mL and $AUC_{0-\infty}$ 1364 (1048) h·μg irinotecan/mL (for irinotecan); or (for SN-38), Cmax 5.4 (3.4) μg SN-38 (as free base anhydrous)/mL; $AUC_{0-\infty}$ 620 (329) h·ng SN-38/mL.

[0023] Unless otherwise indicated, liposomal preparations can comprise (e.g., spherical or substantially spherical) vesicles with at least one lipid bilayer, and may optionally include a multilamellar and/or unilamellar vesicles, and vesicles that encapsulate and/or do not encapsulate pharmaceutically active compounds (e.g., camptothecins) and/or trapping agent(s). For example, unless otherwise indicated, a pharmaceutical liposomal preparation comprising camptothecin liposomes may optionally include liposomes that do not comprise a camptothecin compound, including a mixture of unilamellar and multilamellar liposomes with or without camptothecin compound(s) and/or trapping agent(s).

BRIEF DESCRIPTION OF THE FIGURES

[0024] FIG. 1A shows a schematic of an irinotecan liposome which encapsulates an aqueous space containing irinotecan in a gelated or precipitated state as the sucrose octasulfate salt.

[0025] FIG. 1B shows an equatorial cross section of the irinotecan liposome in FIG. 1A.

[0026] FIG. 2A is a graph of the Stability Ratio values versus the relative amounts of lyso-PC (mol%) of liquid irinotecan liposome compositions after 9 months of storage at 4 °C, the liposome compositions having the designated pH values after manufacture but prior to storage.

[0027] FIG. 2B is a graph of the Stability Ratio values versus the relative amounts of lyso-PC (mol%) of liquid irinotecan liposome compositions after 6 months of storage at 4 °C, the liposome compositions having the designated pH values after manufacture but prior to storage.

[0028] FIG. 2C is a graph of the Stability Ratio values versus the relative amounts of lyso-PC (mol%) of liquid irinotecan liposome compositions after 6 months of storage at 4 °C, the liposome compositions having the designated pH values after manufacture but prior to storage.

[0029] FIG. 3A is a graph of the relative amounts of lyso-PC (mol%) versus the months of storage at 4 °C of two irinotecan liposome compositions having a Stability Ratio of 1047 and a pH of 6.5.

[0030] FIG. 3B is a graph of the relative amounts of lyso-PC (mol%) versus the months of storage at 4 °C of two irinotecan liposome compositions having Stability Ratios of 992 and 942, respectively, and a pH after manufacture but prior to storage of 6.5.

[0031] FIG. 3C is a graph of the relative amounts of lyso-PC (mol%) versus the months of storage at 4 °C of an irinotecan liposome composition having a Stability Ratio of 785 and a pH after manufacture but prior to storage of 6.5.

[0032] FIG. 3D is a graph of the relative amounts of lyso-PC (mol%) versus the months of storage at 4 °C of two irinotecan liposome compositions having a Stability Ratio of about 724, prepared using TEA_8SOS at a sulfate group concentration of 0.65 M, and having a pH after manufacture but prior to storage of 6.5.

[0033] FIG. 4A is a graph of the relative amounts of lyso-PC (mol%) versus the months of storage at 4 °C of three irinotecan liposome compositions having a Stability Ratio of about 1047 and a pH after manufacture but prior to storage of 7.25. Liposome sample 5 (open square) was prepared at an irinotecan moiety concentration equivalent to that provided by 5

mg/mL irinotecan hydrochloride trihydrate, while liposome sample 13 (closed triangle) was likewise prepared at 20 mg/mL irinotecan hydrochloride trihydrate. The liposomes in samples 13 were prepared in the same way as in sample 5, but liposomal components (i.e., phospholipids, cholesterol, irinotecan and sucrosofate) per milliliter in the final liposome composition were increased fourfold compared to sample 5.

[0034] FIG. 4B is a graph of the relative amounts of lyso-PC (mol%) versus the months of storage at 4 °C of two irinotecan liposome compositions having a Stability Ratio of about 1047 and pH values after manufacture but prior to storage of 7.25 and 7.5.

[0035] FIG. 4C is a graph of the relative amounts of lyso-PC (mol%) versus the months of storage at 4 °C of two irinotecan liposome compositions having a Stability Ratio of about 785 and pH values after manufacture but prior to storage of 7.25 and 7.5.

[0036] FIG. 5 is a graph of the concentration of lyso-PC (mg/mL) versus the months of storage at 4 °C of three irinotecan liposome compositions having a Stability Ratio of 1046-1064 and a pH after manufacture but prior to storage of 7.3.

[0037] FIG. 6 is a graph of the concentration of lyso-PC (mg/mL) versus the months of storage at 4 °C in three irinotecan liposome compositions having a Stability Ratio of 1046-1064 and a pH after manufacture but prior to storage of 7.3.

[0038] FIG. 7 is a graph of the estimated rate of lyso-PC (mg/mL/month) formation during storage at 4 °C in irinotecan liposome compositions having various amounts of substituted ammonium (protonated TEA).

[0039] FIG. 8 is a graph of the gram-equivalent amounts of irinotecan and sucrosofate in the precipitate formed by combining, in aqueous solution, irinotecan hydrochloride trihydrate and triethylammonium sucrosofate in various proportions, i.e., in gram-equivalent ratios from 1:9 to 9:1. The x-axis shows the relative gram-equivalent overall amount of triethylammonium sucrosofate (SOS) in the samples, in reference to the gram-equivalent of irinotecan free base anhydrous.

[0040] FIG. 9 shows a graph plotting the average particle size of 12 different irinotecan sucrose octasulfate liposome product lot numbers stored over a period of 12-36 months at 4 °C, with linear regressions to the data obtained for each sample.

[0041] FIG. 10 is a graph of the particle size polydispersity index (PDI) of the irinotecan sucrose octasulfate product lot numbers shown in FIG. 9, with linear regressions to the data obtained for each sample.

[0042] FIG. 11A is a graph of the pH of 13 different irinotecan sucrose octasulfate product lot numbers stored over a period of 12-36 months at 4 °C, with linear regressions to the data obtained for each sample.

[0043] FIG. 11B is a graph of the pH of 16 different irinotecan sucrose octasulfate product lot numbers stored over a period of 12 months at 4 °C, with linear regressions to the data obtained for each sample.

[0044] FIG. 12 is a graph of the concentration of lyso-PC (mg/mL) over 36 months in two irinotecan liposome compositions, and best-fit linear regressions to the respective data points obtained from each irinotecan liposome sample.

[0045] FIG. 13A is a representative chromatogram for Method A at full scale.

[0046] FIG. 13B is a representative chromatogram for Method A at enlarged scale.

DETAILED DESCRIPTION

[0047] Stabilized camptothecin compositions can include liposomes encapsulating one or more camptothecin compound(s). Liposomes can be used for the administration of pharmaceutical drugs, including chemotherapeutic drugs. The present invention provides stabilized phospholipid-containing compositions of camptothecin compounds, e.g., liposomal irinotecan, that generate lower amounts of lyso-phospholipids, e.g., lyso-PC.

[0048] Camptothecin lipomes can encapsulate a camptothecin with a trapping agent inside of a lipid composition (e.g., a phospholipid-containing vesicle). For example, FIG. 1A shows a schematic depicting an irinotecan liposome with a diameter of about 110 nm and having a lipid membrane encapsulating irinotecan. The lipid membrane in this schematic contains the ester-containing phospholipid MPEG-2000-DSPE. The MPEG-2000-DSPE lipids are located in the internal and external lipid layer of the bilayer membrane, as a result of which their PEG moieties are located within the liposome or at the liposomes' external surface, respectively. FIG. 1B shows a cross section of a particular embodiment of the generically depicted liposome in FIG. 1A, in which the unilamellar lipid bilayer membrane includes DSPC, cholesterol, and MPEG-2000-DSPE and encapsulates irinotecan sucrose octasulfate.

[0049] It has now been found that novel stabilized irinotecan liposome compositions comprising ester-containing phospholipids can be made that have low levels of lyso-PC even after extended storage at 2-8 °C, such as at 4 °C, including liposomes that encapsulate irinotecan sucrose octasulfate (SOS) (irinotecan-SOS liposomes) and have significantly reduced lyso-PC formation during refrigerated storage. The present invention is based in part on a number of unexpected observations. First, irinotecan-SOS liposome compositions

surprisingly have substantially less lyso-PC during refrigerated storage when the amount of encapsulated irinotecan is increased relative to the amount of co-encapsulated SOS trapping agent. Second, irinotecan-SOS liposome compositions surprisingly have less lyso-PC during refrigerated storage when the pH of the aqueous medium containing the irinotecan-SOS liposomes after manufacture but prior to storage is above 6.5. Third, irinotecan-SOS liposome compositions surprisingly have less lyso-PC when the amount of residual liposomal trapping agent ammonium/substituted ammonium cation assayed in the composition is below 100 ppm.

Constituent Lipids of Liposomal Camptothecin Compositions

[0050] A variety of lipids, especially phospholipids, are known in the art that can be constituents of liposomes, such as phosphatidyl ethanolamine, and phosphatidyl serine, and it is within the skill in the art to make liposomes with other such phospholipids. In some embodiments, liposomes of the present inventions are composed of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE). Below are described preferred embodiments regarding the lipids present in liposome preparations disclosed herein.

[0051] The liposomal components can be selected to produce the liposomal bilayer membrane which forms unilamellar and/or multilamellar vesicles encapsulating and retaining the active substance until it is delivered to the tumor site. Preferably, the liposome vesicles are unilamellar. The liposomal components are selected for their properties when combined to produce liposomes capable of actively loading and retaining the active substance while maintaining low protein binding in vivo and consequently prolonging their circulation lifetime.

[0052] DSPC is preferably the major lipid component in the bilayer of the liposome encapsulating irinotecan (e.g., comprising 74.4% of total weight of all lipid ingredients). DSPC has a phase transition temperature (Tm) of 55 °C.

[0053] Cholesterol can preferably comprise about 24.3% of total weight of all lipid ingredients. It can be incorporated to in an amount effective to stabilize liposomal phospholipid membranes so that they are not disrupted by plasma proteins, to decrease the extent of binding of plasma opsonins responsible for rapid clearance of liposomes from the circulation, and to decrease permeability of solutes/drugs in combination with bilayer forming phospholipids.

[0054] MPEG-2000-DSPE can preferably comprise about 1.3% of total weight of all lipid bilayer constituents. Its amount and presence on the surface of the irinotecan liposome can be selected to provide a minimal steric barrier preventing liposome aggregation. The MPEG-2000-DSPE coated liposomes of the present invention are shown to be stable with respect to size and drug-encapsulation.

[0055] In some embodiments, the lipid membrane of the liposome preparation is preferably composed of the following ingredients: 1, 2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE), in the ratio of approximately one polyethylene glycol (PEG)-modified phospholipid molecule for every 200 non-PEG-phospholipid molecules.

[0056] In preferred embodiments, liposomes of the present invention are made from a mixture of DSPC, cholesterol, and MPEG-2000-DSPE combined in a 3:2:0.015 molar ratio. In preferred embodiments, liposome preparations of the present invention include 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) at a concentration of about 6.81 mg/mL, cholesterol at a concentration of about 2.22 mg/mL, and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE) at a concentration of about 0.12 mg/mL.

[0057] In more preferred embodiments, liposome preparations of the present invention include 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) at a concentration of 6.81 mg/mL, cholesterol at a concentration of 2.22 mg/mL, and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE) at a concentration of 0.12 mg/mL.

Camptothecin Composition Trapping Agents

[0058] In some embodiments, the liposomes of the present invention encapsulate one or more agents that trap the pharmaceutical drug within liposomes (hereafter referred to as trapping agents). The trapping agent preferably comprises a polyanionic compound with a plurality of negatively charged groups, or comprises a combination of two or more different such compounds. In non-limiting examples, the polyanion trapping agent is a divalent anion, a trivalent anion, a polyvalent anion, a polymeric polyvalent anion, a polyanionized polyol, or a polyanionized sugar. In the context of the present invention, the polyanionic trapping agent can be a polyanionized polyol or sugar, such as a polyol or a sugar having its hydroxyl groups completely or partially modified or replaced with anionic groups (anionized). In a non-limiting example, polyanionized polyol or polyanionized sugar can include a polyol moiety

or a sugar moiety along with anionic groups linked thereto. Preferably, at least one anionic group of a polyanionized sugar or polyanionized polyol trapping agent is more than 50% ionized in a pH range of pH 3-12, preferably pH 6.5-8, when in an aqueous medium, or, alternatively, the anionic group(s) has a pKa of 3 or less, preferably of 2 or less. In a preferred embodiment, the trapping agent contains sulfate moieties having a pKa of 1.0 or less. In a non-limiting example, a polyanion trapping agent can have a charge density of at least two, three, or four negatively charged groups per unit, e.g., per carbon atom or ring in a carbon chain or per monosaccharide unit in a sugar.

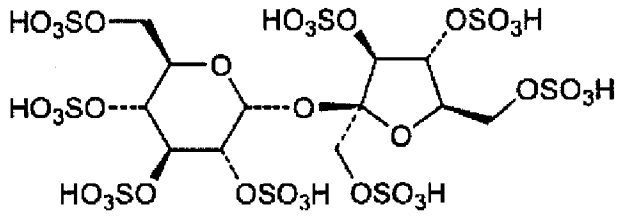
[0059] In some embodiments of the present invention, the release rate of the liposome composition can be increased by using as a trapping agent a mixture of polyanionized sugar or polyanionized polyol with one or more other monovalent or polyvalent anions, e.g., chloride, sulfate, phosphate, etc. In another non-limiting example of increasing the release rate of the extended release composition, mixtures of different polyanionized sugars and/or polyanionized polyols with various degrees of polyanionization are being used as trapping agent.

[0060] In some embodiments, the degree of polyanionization inside the liposomes of the present invention is above 90%, or above 99%, or between 0.1% to 99%, 10% to 90%, or 20% to 80% of the total anion(s) inside the liposomes, e.g., with a liposome-entrapped camptothecin or camptothecin-derivative compound.

[0061] In some embodiments, the trapping agent is a sulfated sugar and/or polyol. Exemplary sulfated sugar of the present invention is sulfated sucrose including, without limitation, sucrose hexasulfate, sucrose heptasulfate, and sucrose octasulfate (See Ochi. K., et al., 1980, Chem. Pharm. Bull., v. 28, p. 638-641). Similarly, reaction with phosphorus oxychloride or diethylchlorophosphate in the presence of base catalyst results in polyphosphorylated polyols or sugars. Polyphosphorylated polyols are also isolated from natural sources. For example, inositol polyphosphates, such as inositol hexaphosphate (phytic acid) can be isolated from corn. A variety of sulfated, sulfonated, and phosphorylated sugars and polyols suitable to practice the present invention are disclosed, e.g., in U.S. Pat. No. 5,783,568, which is incorporated herein by reference in its entirety. Complexation of polyols and/or sugars with more than one molecule of boric acid also results in a polyanionized (polyborated) product. Reaction of polyols and/or sugars with carbon disulfide in the presence of alkali results in polyanionized (polydithiocarbonated, polyxanthogenate) derivatives. A polyanionized polyol or sugar derivative can be isolated in the form of a free acid and neutralized with a suitable base, for example, with an alkali metal hydroxide,

ammonium hydroxide, or preferably with a substituted amine, e.g., amine corresponding to a substituted ammonium of the present invention, in a neat form or in the form of a substituted ammonium hydroxide providing for a polyanionic salt of a substituted ammonium of the present invention. Alternatively, a sodium, potassium, calcium, barium, or magnesium salt of a polyanionized polyol/sugar can be isolated and converted into a suitable form, e.g., a substituted ammonium salt form, by any known method, for example, by ion exchange. Non-limiting examples of sulfated sugar trapping agents are sulfated sucrose compounds including, without limitation, sucrose hexasulfate, sucrose heptasulfate, and sucrose octasulfate (SOS). Exemplary polyol trapping agents include inositol polyphosphates, such as inositol hexaphosphate (also known as phytic acid or IHP) or sulfated forms of other disaccharides.

[0062] In a preferred embodiment of the present invention, the trapping agent is a sulfated polyanion, a non-limiting example of which is sucrose octasulfate (SOS). Sucrosofate is also referred to as sucrose octasulfate or sucrooctasulfate (SOS). Methods of preparing sucrosofate in the form of various salts, e.g., ammonium, sodium, or potassium salts, are well known in the field (e.g., US Pat. 4,990,610, incorporated by reference herein in its entirety). Sucrose octasulfate (also referred to as sucrosofate), is a fully substituted sulfate ester of sucrose having, in its fully protonated form, the structure of formula (II):

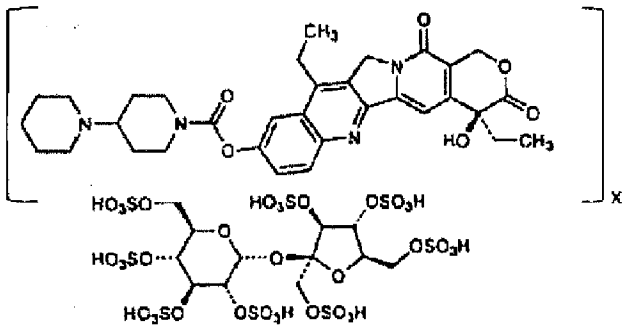


(II).

[0063] Methods of preparing sucrosofate in the form of various salts, e.g., ammonium, sodium, or potassium salts, are well known in the field (see, e.g., U.S. Patent No. 4,990,610, which is incorporated by reference herein in its entirety). Likewise sulfated forms of other disaccharides, for example, lactose and maltose, to produce lactose octasulfate and maltose octasulfate, are envisioned.

[0064] In some embodiments, the liposome formulations of the present invention comprise a camptothecin compound such as irinotecan or topotecan and an anionic trapping agent such as SOS. The liposomes of the present invention preferably include the camptothecin compound in a stoichiometric ratio with the anionic trapping agent. For example, an irinotecan liposome formulation can encapsulate irinotecan and a sucrose octasulfate in about

an 8:1 mole ratio. Stabilized compositions of liposomes can encapsulate the irinotecan composition of formula (I), where x is about 8:



(I).

The liposomal irinotecan can comprise the composition of formula (I) encapsulated in liposomes. Preferably, the composition of formula (I) is formed (e.g., precipitated) within liposomes comprising cholesterol and one or more phospholipid(s) (e.g., including PEG-containing phospholipid(s)). For example, the compound of formula (I) can be formed within the liposomes by reacting (1) a camptothecin compound(s) (e.g., irinotecan, topotecan, and the like) with (2) liposomes encapsulating a polysulfated anionic trapping-agent (e.g., sucrose octasulfate), in a process that forms a stabilized liposomal irinotecan composition. Preferably, the liposomal irinotecan composition has a pH greater than 6.5 (e.g., 7.0-7.5, including 7.25, 7.3 and 7.5).

[0065] Preferred stabilized camptothecin compositions include liposomal irinotecan.

[0066] Stabilized camptothecin compositions include high-density camptothecin compound(s) liposome formulations containing irinotecan or a salt thereof at an irinotecan moiety concentration equivalent to that provided by from 4.5 to 5.5 mg/mL irinotecan hydrochloride trihydrate (i.e., 3.9-4.8 mg/mL irinotecan free base anhydrous), and contain DSPC at a concentration of from 6.13 to 7.49 mg/mL (preferably about 6.81 mg/mL), cholesterol at a concentration of from 2-2.4 mg/mL (preferably about 2.22 mg/mL), and MPEG-2000-DSPE at a concentration of 0.11-0.13 mg/mL (preferably about 0.12 mg/mL), and are characterized by the presence of low amounts of lyso-PC, if any, during refrigerated storage (2-8°C), while also providing suitable amounts of the camptothecin compound(s), preferably in a more potent lactone form. The present invention includes pharmaceutical camptothecin compound(s) liposome compositions that can be stored under refrigeration (i.e., at 2-8 °C) for at least the first 6 months, preferably at least the first 9 months, following manufacture without the formation of levels of lyso-PC above 20 mol%. More preferably, the

present invention provides for compositions containing an amount of irinotecan moiety equivalent to that provided by between 4.7-5.3 mg/mL irinotecan hydrochloride trihydrate (i.e., 4.1-4.6 mg irinotecan moiety free anhydrous base) (the irinotecan can be present as a sucrose octasulfate salt encapsulated within the liposomes), along with (DSPC) at 6.4-7.2 mg/mL, cholesterol at 2.09-2.35 mg/mL, and MPEG-2000-DSPE at about 0.113-0.127 mg/mL that contains no more than 20 mol% lyso-PC at 6 or 9 months when stored at 2-8 °C, or no more than 2 mg/mL lyso-PC at 21 months when stored at 2-8 °C.

Calculation of Irinotecan/Sulfate Compound Gram-Equivalent Ratio (ER)

[0067] An irinotecan/sulfate compound gram-equivalent ratio (ER), can be calculated for each irinotecan liposome preparation by determining molar amounts of liposomally co-encapsulated irinotecan (I) and sulfate compound (S) per unit (e.g., 1 mL) of the liposome composition, and using the formula: ER = I/(SN), where N is valency of the sulfate compound anion (e.g., for sucrosofate N is 8, and for free sulfate, $SO4^{2-}$, N is 2). For example, the liposomal irinotecan sucrosofate composition that contains 7.38 mM irinotecan and 1.01 mM sucrosofate (N=8) would have the ER of 7.38/(1.01x8)=0.913. Preferably, the sulfate compound (S) is sucrose octasulfate, containing 8 sulfate moieties per mol of SOS. The liposomal composition will have a pH of from 7.1 to 7.5 and have one of the following ER ranges: preferably 0.85 to 1.2, 0.85-1.1 or most preferably from 0.9 to 1.05, such as about 1.02. Alternatively the liposomal composition will have an irinotecan moiety amount equivalent to that provided by 500 g (±10%) irinotecan free anhydrous base per mol phospholipid and nd have one of the following ER ranges: preferably 0.85 to 1.1, most preferably from 0.9 to 1.05, such as about 1.02.

pH of Stabilized Camptothecin Composition

[0068] The pH of the liposomal composition can be adjusted or otherwise selected to provide a desired storage stability property (e.g., to reduce formation of lyso-PC within the liposome during storage at 4 °C over 180 days), for example by preparing the composition at a pH of about 6.5-8.0 or any suitable pH value there between (including, e.g., 7.0-8.0, and 7.25). In some embodiments, the pH is about 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, or 8.0. Irinotecan liposomes with particular pH values, irinotecan moiety equivalent to that provided by irinotecan free anhudrous base concentration (mg/mL) and various concentrations of sucrose octasulfate were prepared as provided in more detail as described herein. More preferably, the pH after manufacture and before storage is between

7.1 and 7.5 and even more preferably between about 7.2 and 7.3, and most preferably about 7.25. The pH can be adjusted by standard means, e.g. using 1N HCl or 1N NaOH, as appropriate.

[0069] In some embodiments of the present invention, the pH of the liposomal irinotecan preparation after manufacture but prior to storage is above 6.5, preferably between to 7.2 and 7.3. In some embodiments of the present invention, the pH is from 7.2 to 7.5.

Compound Gram-Equivalent Ratio ("ER") of Stabilized Camptothecin Compositions

[0070] Stabilized liposomal camptothecin compositions can have a pH greater than 6.5 and comprise liposomes encapsulating irinotecan and a sulfate polyanionic trapping agent having an irinotecan/sulfate compound gram-equivalent ratio ("ER") that is greater than 0.9 (e.g., 0.9-1.1). The ER can be calculated for an irinotecan SOS liposome preparation by determining molar amounts of liposomally co-encapsulated irinotecan (I) and sulfate compound (S) per unit (e.g., 1 mL) of the liposome composition, and using the formula: ER = I/(SN), where N is valency of the sulfate compound anion (e.g., for sucrosofate N is 8, and for free sulfate, SO_4^{2-}, N is 2), I is the concentration of encapsulated irinotecan in the liposome irinotecan composition, and S is the concentration of the sulfate groups of encapsulated sucrose octasulfate in the liposomal irinotecan composition. Preferably, the sulfate compound (S) is sucrose octasulfate, containing 8 sulfate moieties per mol of SOS. ,

[0071] While the direct determination of the encapsulated sucrose octasulfate sulfate groups concentration in the liposomal irinotecan composition (S·N) is preferred, S·N can be determined from the liposome phospholipid concentration (P, mol/L), SOS sulfate groups concentration in the inner space of the liposome (the SOS sulfate groups concentration in the solution used to prepare a trapping agent liposome; parameter B, see Stability Ratio definition herein), and the liposome internal (entrapped) volume, that is, the volume sequestered within the inner space of the liposome vesicles, per unit of liposome phospholipid (Ve, L/mol phospholipid):

$$S \cdot N = P \cdot Ve \cdot B$$

[0072] By way of example, for a phosphatidylcholine-cholesterol liposome obtained by extrusion via 100-nm polycarbonate filters, entrapped volume can be close to 1.7 L/mol phospholipid (Mui, et al. 1993, Biophys.J., vol 65, p. 443-453). In this case, quantitative loading of irinotecan (molecular weight 586.7) into the SOS-encapsulating liposomes at 471 g/mol phospholipid and the SOS sulfate groups concentration of 0.45 M, will result in an ER of

$$(471/586.7)/(1.7 \cdot 0.45) = 1.049$$

[0073] While at the SOS concentration of 0.65 M sulfate groups, the ER will be:

$$(471/586.7)/(1.7 \cdot 0.65) = 0.726$$

[0074] Similarly, quantitative loading of irinotecan (molecular weight 586.7) into the SOS-encapsulating liposomes at 500 g(±10%) /mol phospholipid and the SOS sulfate groups concentration of 0.45 M, will result in an ER of about 1.11, while at the SOS concentration of 0.65 M sulfate groups, the ER will be about 0.77.

Preparing Stabilized Camptothecin Compositions

[0075] The stabilized camptothecin compositions can comprise camptothecin liposomes. Liposomes have been used for the administration of pharmaceutical drugs, including chemotherapeutic drugs. Various technology relating to drug-encapsulating liposomes and methods of making the same are generally known in the art and are therefore not further described herein in any detail. See, e.g., U.S. Patent No. 8,147,867, which is incorporated herein by reference in its entirety.

[0076] In some embodiments, liposomes encapsulating one or more camptothecin compound(s) within a vesicle comprises at least one phospholipid. The camptothecin compound can, for example, be loaded or otherwise entrapped within the liposome in a multi-step process comprising (a) forming a trapping agent liposome encapsulating the anionic trapping agent and a cation within a liposome vesicle comprising phospholipid(s), and (b) subsequently contacting the trapping agent liposome with the camptothecin compound(s) under conditions effective to load the camptothecin compound(s) into the trapping agent liposome and retain the camptothecin compound inside the liposome with the trapping agent to form the camptothecin liposomes.

[0077] The camptothecin compound(s) can be loaded into the trapping agent liposomes using a gradient across the liposome membrane, causing the camptothecin compound(s) to enter the trapping agent liposomes to form the camptothecin liposomes. Preferably, the trapping agent liposomes have a transmembrane concentration gradient of a membrane-traversing cation, such as ammonium or substituted ammonium, effective to result in the exchange of the ammonium/substituted ammonium in the trapping agent liposomes for the camptothecin compound(s) when heated above the phase transition temperature of the lipid components of the liposomes. Preferably, the trapping agent has a higher concentration in the trapping agent liposome than in the media surrounding it. In addition, the trapping agent liposomes can include one or more trans-membrane gradients in addition to the gradient

created by the ammonium/substituted ammonium cation. For example, the liposomes contained in the trapping agent liposome composition can additionally or alternatively include a transmembrane pH gradient, ion gradient, electrochemical potential gradient, and/or solubility gradient.

[0078] In some embodiments, the trapping agent used for the preparation of liposomes (e.g., SOS and/or another sulfated polyol trapping agent, including acceptable salts thereof) has a concentration of 0.3-08, 0.4-.05, 0.45-0.5, 0.45-.0475, 0.45-0.5, 0.3, 0.4, 0.45, 0.475, 0.5, 0.6, 0.7, or 0.8 M sulfate groups, e.g. these specific values ±10%. In a preferred embodiment, the trapping agent used for the preparation of liposomes is SOS and has a concentration of about 0.45 or about 0.475 M sulfate groups. In a more preferred embodiment, the trapping agent used for the preparation of liposomes is SOS and has a concentration of 0.45 M or 0.475 M sulfate groups.

[0079] Preferably, the camptothecin compound(s) is loaded into the trapping agent liposome by incubating the camptothecin compound(s) with the trapping agent liposomes in an aqueous medium at a suitable temperature, e.g., a temperature above the primary phase transition temperature of the component phospholipids during loading, while being reduced below the primary phase transition temperature of the component phospholipids after loading the camptothecin compound(s), preferably at about room temperature. The incubation time is usually based on the nature of the component lipids, the camptothecin compound(s) to be loaded into the liposomes, and the incubation temperature. Typically, the incubation times of several minutes (for example 30-60 minutes) to several hours are sufficient.

[0080] Because high entrapment efficiencies of more than 85%, typically more than 90%, are achieved, there is often no need to remove unentrapped entity. If there is such a need, however, the unentrapped camptothecin compound(s) can be removed from the composition by various means, such as, for example, size exclusion chromatography, dialysis, ultrafiltration, adsorption, and precipitation.

[0081] In some embodiments, the camptothecin liposomes are irinotecan liposomes. The irinotecan liposomes can be prepared by a process that includes the steps of (a) preparing a liposome containing triethylamine (TEA) as a triethylammonium salt of sucrosofate (TEA-SOS), and (b) subsequently contacting the TEA-SOS liposome with irinotecan under conditions effective for the irinotecan to enter the liposome and to permit a corresponding amount of TEA to leave the liposome (thereby exhausting or reducing the concentration gradient of TEA across the resulting liposome).

Extraliposomal Ionic Strength During Drug Loading of Camptothecin Liposomes

[0082] In some embodiments of the present invention, the camptothecin loading of the liposomes is conducted in an aqueous solution at the ionic strength of less than that equivalent to 50 mM NaCl, or more preferably, less than that equivalent to 30 mM NaCl. After drug loading, a more concentrated salt solution, e.g., NaCl solution, may be added to raise the ionic strength to higher than that equivalent to 50 mM NaCl, or more preferably, higher than that equivalent to 100 mM NaCl, preferably equivalent to between about 140-160 mM NaCl.

Trapping Agent Cations

[0083] The cation of the present invention can be encapsulated into the trapping agent liposomes in an amount effective to provide for the loading of the camptothecin compound(s) into the trapping agent liposomes, when heated above the phase transition temperature of the lipid components as described above. The cations are selected so that they can leave the trapping agent liposomes during the loading of the camptothecin compound(s) into the liposomes. Extra-liposomal cations can be removed after the preparation of the liposomes loaded with camptothecin compound(s).

[0084] In some embodiments of the present invention, the cation in the liposome together with the trapping agent is a substituted ammonium compound. In some embodiments of the invention, the substituted ammonium compound has a pKa of at least about 8.0. In some embodiments of the invention, the substituted ammonium compound has a pKa of at least about 8.0, at least about 8.5, at least about 9.0, at least 9.5, at least 10.0, at least 10.5, or at least 11.0 as determined in an aqueous solution at ambient temperature. In some embodiments of the invention, the substituted ammonium compound has a pKa of about 8.0-12.0, about 8.5.-11.5, or about 9.0-11. In a preferred embodiment, the pKa is about the pKa of TEA, or about the pKa of DEA.

[0085] Non-limiting examples of such substituted ammonium compounds are compounds of the formula: $N(R_1)(R_2)(R_3)(R_4)^+$ where each of R_1, R_2, R_3, and R_4 are independently a hydrogen or an organic group having up to 18 total carbon atoms, and where at least one of R_1, R_2, R_3, and R_4 is an organic group that is a hydrocarbon group having up to 8 carbon atoms, which can be an alkyl, alkylidene, heterocyclic alkyl, cycloalkyl, aryl, alkenyl, or cycloalkenyl group or a hydroxyl-substituted derivative thereof, optionally including within its hydrocarbon moiety one or more S, O, or N atom(s) forming an ether, ester, thioether,

amine, or amide bond. The substituted ammonium may be a sterically hindered ammonium compound (e.g., having at least one of the organic groups with a secondary or tertiary carbon atom directly linked to the ammonium nitrogen atom). Also, at least one of R_1, R_2, R_3 and R_4, must be hydrogen. Preferably, the substituted ammonium cation is triethylammonium (protonated TEA) or diethylammonium (protonated DEA).

[0086] The concentration of the substituted ammonium cation within the trapping agent liposome can be reduced as the camptothecin compound is loaded into the liposomes encapsulating the anionic trapping agent under conditions effective to form the camptothecin compound liposomes. The liposomes of the present invention can include an anionic trapping agent and an ammonium or substituted ammonium cation that is subsequently removed and/or replaced by the camptothecin compound loaded into the liposome in a subsequent drug loading step.

[0087] In a preferred embodiment, the concentration of the ammonium or substituted ammonium cation within the camptothecin compound liposomes is low enough to provide low amounts of lyso-PC after refrigerated storage for prolonged periods of camptothecin liposome preparations that contain phospholipids. For example, as discussed in Example 3, including the data in FIG. 7, reduction in the amount of lyso-PC formation was observed in irinotecan SOS liposome preparations having less than about 100 ppm of the substituted ammonium cation, preferably between 20 and 80 ppm, preferably less than about 50 ppm, even more preferably less than about 40 ppm, still more preferably less than 30 ppm.

[0088] In some embodiments, the irinotecan SOS liposomes (such as Samples 24-29; Table 10 of the Examples) comprise less than 100 ppm, or about 15-100 ppm substituted ammonium SOS trapping agent counter ion. In some embodiments, the irinotecan SOS liposomes (such as Samples 24-29; Table 10 of the Examples) comprise about 15-80 ppm substituted ammonium. In some embodiments, irinotecan SOS liposomes comprise about 40-80 ppm substituted ammonium. In some embodiments, the irinotecan SOS liposomes (such as Samples 24-29; Table 10 of the Examples) comprise about 80-100 ppm substituted ammonium. In a preferred embodiment, the substituted ammonium present at any of the above-mentioned ppm concentrations is derived from TEA or DEA.

Stability Ratio of Stabilized Camptothecin Compositions

[0089] When phospholipid-based camptothecin-containing liposomes are made by reacting (1) a camptothecin drug with (2) liposomes encapsulating a polysulfated anionic trapping-agent, the stability of the resulting drug-loaded liposomes depends on the ratio of the

camptothecin, an anionic trapping agent and liposome-forming phospholipids as defined by a Stability Ratio of at least about 950, as defined below. The Stability Ratio depends on the initial concentration of sulfate groups in the trapping-agent-liposomes and the ratio of camptothecin encapsulated to phospholipid in the liposomes. As used herein, the Stability Ratio ("SR") is defined as follows:

$$SR = A/B,$$

where:

a. A is the amount of irinotecan moiety encapsulated in trapping agent liposomes during the drug loading process, in grams equivalent to the irinotecan free anhydrous base, per mole of phospholipid in the composition; and

b. B is the concentration of sulfate groups in the sucrosofate (or other trapping agent) solution used to make the trapping agent liposomes, expressed in mole/L (based on the concentration of sulfate groups).

[0090] With respect to the determination of the Stability Ratio, the number of moles of phospholipid in the liposome preparation is determined by assay, such as described in the Examples. The irinotecan moiety amount (A above) is calculated accordingly for conducting liposome loading.

[0091] With respect to the determination of the Stability Ratio, the concentration B of sulfate groups in the sucrosofate (or other trapping agent) solution, expressed in mole/L, is calculated as the concentration of sucrosofate (or other trapping agent disclosed herein) (in mole/L) in the solution that is added to lipids (which are typically dissolved in alcohol, typically in a volume that is 10% or less than the volume of the trapping agent solution added to the lipids). Thus for sucrosofate, the concentration B of sulfate groups is the concentration of sucrosofate multiplied by 8 (i.e., the number of sulfate groups in one sucrosofate molecule), or multiplied in accordance with the number of sulfate groups of the particular trapping agent used. (See Example 1.)

[0092] In some embodiments of the present invention, the Stability Ratio and the pH are both increased to greater than 6.5. Thus, in certain preferred embodiments of the present invention, the Stability Ratio is 942-1130, and the pH is from 7.2 to 7.5, and the irinotecan and SOS trapping agent are present in the liposome composition in an about 8:1 molar ratio. Preferably the Stability Ratio is 942-1130, the pH is about 7.25, and the irinotecan composition and SOS trapping agent are present in the liposome in an 8:1 molar ratio. The amount of lyso-PL, and in particular, lyso-PC, in formulations of liposomes encapsulating other camptothecin compounds may be controlled in a similar fashion.

[0093] For example, the novel stabilized irinotecan liposome preparations can have 80% less lyso-PC compared to irinotecan SOS liposomes prepared according to other processes (e.g., 80% less lyso-PC than observed in comparative Sample 12 after 9 months of refrigerated storage). A (comparative) liposomal irinotecan of sample 12 was prepared with a Stability Ratio of about 724 by heating a lipid mixture having a 3:2:0.015 mole ratio of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE), in the presence of triethylamine (TEA) and sucrose octasulfate ("SOS" or "sucrosofate") in a 8:1 mole ratio [$(TEA)_8SOS$] at a sulfate group concentration of 0.65 M to generate TEA-SOS trapping agent liposomes. After removal of $(TEA)_8SOS$ not encapsulated in the TEA-SOS trapping agent liposomes, irinotecan was loaded into the resulting preparation containing the TEA-SOS trapping agent liposomes using a solution of irinotecan under conditions resulting in the removal of TEA and loading into the liposomes a total amount of irinotecan provided by 500 g (±10%) of irinotecan anhydrous free base per mole of phospholipids in the TEA-SOS trapping agent liposome preparation. The pH of the irinotecan liposome composition was 6.5 (measured in accordance with the subsection "pH Measurements" in the Examples section herein), with 4.3 mg of irinotecan moiety in the irinotecan liposomes per mL of the irinotecan liposome composition. These phosphatidylcholine-containing liposomal irinotecan compositions generated levels of lyso-PC in excess of 30 mol% (with respect to the total amount of phosphatidylcholine in the irinotecan liposome compositions) during 3 months (and over 35mol% lyso-PC generated during 9 months) of refrigerated storage (2-8°C).

Calculation of Stability Ratios and Lyso-PC Amounts in Exemplary Embodiments

[0094] A series of different irinotecan liposome preparations were made according to the methods described herein (additional experimental details for preparation and characterization of each sample are included below in the Examples). The amount of lyso-PC measured in each of the irinotecan liposome preparations is summarized in Table 1A (lyso-PC measurements taken after 9 months of refrigerated storage) and Table 1B (lyso-PC measurements taken after 6 months of refrigerated storage, for a sub-set of the samples listed in Table 1A). Each irinotecan liposome preparation contained unilamellar bilayer liposomes of about 110±20 nm, preferably 110±10 nm in diameter encapsulating irinotecan with a sucrose octasulfate trapping agent. The liposomes were formed from a mixture of DSPC, cholesterol, and MPEG-2000-DSPE having a 3:2:0.015 molar ratio and then loaded with irinotecan at a concentration of about 471 g irinotecan moiety (irinotecan or a salt thereof

providing an amount of irinotecan moiety equivalent to 500 g (±10%) of irinotecan HCl anhydrous) per mole phospholipid. Each irinotecan liposome preparation contained different amounts of the SOS trapping agent and were formulated at different pH values. The amount of lyso-PC was measured in each irinotecan liposome preparation at various times, including a measurement of all samples after 9 months of continuous refrigerated storage (at 4 °C). All samples in Table 1A were loaded using a protonated TEA counter-ion for SOS (i.e., loading irinotecan into liposomes encapsulating various concentrations of TEA_8SOS, as specified in Table 1A).

Table 1A: *Irinotecan Liposome Stability Ratio and Lyso-PC (after 9 months at 4 °C)*[a]

Sample	Molar (M) concentration of sulfate groups in the sucrosofate entrapped in the liposomes	Stability Ratio	pH	[mol% Lyso-PC] at 9 mos.
Comparator (12)	0.65	724	6.5	35.4
1	0.45	1047	6.5	25.4
2	0.475	992	6.5	23.6
3	0.5	942	6.5	35.7
4	0.6	785	6.5	35.8
5	0.45	1047	7.25	11.1
6	0.45	1047	6.5	17.4
7	0.45	1047	7.25	8.1
8	0.45	1047	7.5	7.1
9	0.6	785	6.5	34.7
10	0.6	785	7.25	29
11	0.6	785	7.5	28.7
13	0.45	1047	7.25	13.8
14	0.65	724	6.5	32.1

[a] Measured according to Method B, as described herein.

[0095] FIG. 2A shows a plot depicting the amount of lyso-PC measured in each sample in Table 1A after 9 months of storage at 4 °C. Sample 12 is labeled as a Comparator in Table 1A and FIG. 2A. Samples having both a Stability Ratio greater than about 900 and a pH of greater than 6.5 (e.g., 7.25 and 7.5) contained less than 20 mol% lyso-PC after 9 months of refrigerated storage at 4 °C. FIG. 2C is a graph of the Stability Ratio values versus the relative amounts of lyso-PC (mol%) of liquid irinotecan liposome compositions after 6 months of storage at 4 °C (data in Table 6). The data points indicated with open circles correspond to irinotecan samples having a pH of greater than 6.5 (7.25 or 7.5) measured after manufacture but prior to storage. The data points indicated with diamonds correspond to irinotecan

samples having a pH of 6.5, measured after manufacture but prior to storage. The Stability Ratio was calculated as defined herein, during the manufacture of each sample. The mol% lyso-PC was measured after the first 6 months of storage following the manufacture of each sample.

Table 1B: *Irinotecan Liposome Stability Ratio and Lyso-PC (after 6 months at 4 °C)*[b]

Sample	Molar (M) concentration of sulfate groups in the sucrosofate entrapped in the liposomes	Stability Ratio	pH	[mol% Lyso-PC] at 6 mos.
1	0.45	1047	6.5	19.5
2	0.475	992	6.5	17
3	0.5	942	6.5	26.5
4	0.6	785	6.5	30.2
5	0.45	1047	7.25	7.1
6	0.45	1047	6.5	14.6
7	0.45	1047	7.25	7.4
8	0.45	1047	7.5	5.4
9	0.6	785	6.5	29.8
10	0.6	785	7.25	24.1
11	0.6	785	7.5	22.8
13	0.45	1047	7.25	9.72

[b] Measured according to Method B, as described herein.

[0096] FIG. 2B shows a plot depicting the amount of lyso-PC measured in each sample in Table 1B after 6 months of storage at 4 °C. Samples having both a Stability Ratio greater than about 989 and a pH of greater than 6.5 (e.g., 7.25 and 7.5) contained less than 20 mol% lyso-PC after 6 months of refrigerated storage at 4 °C.

[0097] FIGS. 3A-3D are plots showing the mol% of lyso-PC in irinotecan liposome preparations selected from Table 1A and 1B having a pH of 6.5. Lyso-PC was determined after storage of each sample at 4 °C for 0, 1, 3, 6, 9, and/or 12 months. These plots include a linear regression line to the data, as an estimate for the rate of increase in lyso-PC (mol%) over time in each sample. A summary of the slope, y-intercept, and R^2 values for each FIG. is shown in Table 1C below.

Table 1C: *Mol% of lyso-PC vs. refrigerated storage time (months) at pH 6.5*

FIG.	Sample	Stability Ratio	y-intercept (mol% lyso-PC)	Slope (mol% lyso-PC per month)	R^2
3A	1	1047	2.8	2.6	0.9909
3A	6	1047	4.8	1.5	0.97763
3B	2	992	3.5	2.2	0.9999
3B	3	942	6.8	3.2	0.9996
3C	4	785	11.1	2.8	0.9370
3D	12	724	14.3	2.3	0.6838
3D	14	724	9.6	2.4	0.9096

[0098] In some embodiments, the stability of an irinotecan liposome preparation containing irinotecan SOS encapsulated in liposomes of about 100 nm (e.g., 100 ± 20 nm) in diameter is significantly increased in irinotecan liposomes where the Stability Ratio is greater than 942. By maintaining the constant drug loading ratio of 500 g (±10%) irinotecan moiety (as explained above, based on the free base anhydrous) to total phospholipid, but varying the concentration of SOS trapping agent, the effect of the Stability Ratio on the formation of lyso-PC in the liposome preparation was evaluated. Table 2 provides a summary of the amount of mol% lyso-PC detected in the irinotecan liposome preparations in Table 1 formulated at the same pH as the (comparative) Sample 12 (6.5), but at different concentrations of SOS trapping agent (i.e., at different Stability Ratios). Table 2 illustrates that having a Stability Ratio of greater than 942 as to irinotecan liposomes containing a SOS trapping agent and irinotecan reduce the formation of lyso-PC during refrigerated storage. Reducing the amount of SOS trapping agent (i.e., increasing the Stability Ratio) by up to 30% relative to the Comparator irinotecan liposome preparation resulted in a slight increase in the amount of lyso-PC by about 1% after 9 months of refrigerated storage. However, increasing the amount of SOS trapping agent in an irinotecan liposome preparation having a Stability Ratio of above 942 results in a significant and unexpected decline in the amount of lyso-PC (mol%) present after 9 months of refrigerated storage at 4 °C. For example, a subsequent 5% incremental increase in the Stability Ratio above 942 (i.e., a Stability Ratio of 992 in Sample 2) resulted in a dramatic decrease of the amount of lyso-PC (mol%) present by 34%, compared to Sample 3, equivalent to a 33% decrease in the amount of lyso-PC (mol%) compared to Sample 12 (as measured at 9 months of refrigerated storage at 4 °C). Overall, after 9 months of refrigerated storage at 4 °C, reductions of lyso-PC (mol%) of about 28-51% were achieved by raising the Stability Ratio of irinotecan liposome above 942, compared to Comparator Sample 12. In some embodiments, the irinotecan SOS liposome compositions

have a Stability Ratio of above 942. In preferred embodiments, the irinotecan SOS liposome preparations have a Stability Ratio of 942-1130 or greater (e.g., Stability Ratios of 992-1047).

Table 2: *Irinotecan Liposome Stability Ratio and Lyso-PC (after 9 months at 4 °C, pH 6.5)*

1 Sample	2 Stability Ratio	3 Lyso-PC @ 9mos	4 %SR increase relative to comparator	5 % lyso-PC @ 9mos relative to comparator	6 Incremental %SR increase	7 Incremental % lyso-PC @ 9 months
12 (Comparator)	724	35.4	0	0	0	0
9	785	34.7	+8.3	-2	+8	-2
3	942	35.7	+30	+1	+20	+3
2	992	23.6	+37	-33	+5	-34
6	1047	17.4	+44	-51	+6	-26
1	1047	25.4	+44	-28	+6	+8

[0099] Table 2 illustrates the criticality of having a Stability Ratio of greater than 942 (preferably greater than 950, and most preferably greater than 992) in stabilizing irinotecan liposomes at pH 6.5 containing a SOS trapping agent and irinotecan, to reduce the formation of intra-liposomal lyso-PC during refrigerated storage. Overall, reductions of intra-liposomal lyso-PC of about 28-51% during storage for 6 months at 4 degrees C were achieved by preparing irinotecan liposome compositions at pH 6 having a Stability Ratio above 950 (e.g., 950-1050). Reducing the concentration of SOS trapping agent used in preparing the trapping agent liposomes (i.e., increasing the Stability Ratio) by up to 30% relative to the corresponding concentration of SOS trapping agent used to prepare the Comparator irinotecan liposome preparation (compare samples 3 and 12) resulted in a slight increase in the amount of lyso-PC by about 1% after 9 months of refrigerated storage. However, increasing the amount of SOS trapping agent used to form the trapping agent liposomes prior to irinotecan loading to form an irinotecan liposome preparation having a Stability Ratio of 992 or higher resulted in a significant and unexpected decline in the lyso-PC formation after the first 9 months of refrigerated storage of the resulting irinotecan liposome after manufacturing. For example, the data in Table 2 shows a 5% increase in the Stability Ratio above 942 resulted in a 34% decrease in LysoPC after 9 months of storage at 4 degrees C (Sample 2 compared to Sample 3). Increasing the Stability Ratio from 992 (Sample 2) to 1047 (a 6% increase in SR) resulted in a 26% reduction in Lyso-PC generated after 9 months of storage at 4 degrees C (Sample 6 compared to Sample 2), and an 8% increase in Lyso-PC generated after 9 months of storage at 4 degrees C (Sample 1 compared to Sample 2).

Accordingly, preferred irinotecan SOS liposome compositions have a Stability Ratio of above 1000, including irinotecan SOS liposome preparations with a Stability Ratio of 1000-1200 or greater (e.g., Stability Ratios of 1053-111).

[00100] In some embodiments of the present invention, the stability of an irinotecan liposome preparation containing irinotecan SOS encapsulated in liposomes of about 100±20 nm, preferably 100±10 nm, in diameter is significantly increased by raising the pH of the preparation after manufacture but prior to storage above pH 6.5. By maintaining the constant drug loading ratio of 471 g or 500 g irinotecan moiety (as explained above, based on the free base anhydrous) per mol phospholipid but varying the pH of the final pH of the irinotecan liposome composition, the effect of the pH on the formation of lyso-PC in the liposome preparation was evaluated. Table 3 provides a summary of the amounts of lyso-PC in irinotecan liposome preparations in Table 1 formulated at different pH values. Table 3A reports data from Table 1 for irinotecan liposome preparations, formed by loading liposomes (encapsulating TEA_8SOS at a sulfate group concentration of 0.6 M) with a total of 471 g irinotecan moiety (as explained above, based on the free base anhydrous) per mole of phospholipid (i.e., a Stability Ratio of 471/0.6 or 785). The % change in lyso-PC formation was calculated with respect to both Sample 4 and Sample 9 (both of which had pH 6.5 after manufacture but prior to storage). Table 3B reports data from Table 1 for irinotecan liposome preparations, formed by loading liposomes (encapsulating TEA_8SOS at a sulfate group concentration of 0.45 M) with a total of 471 g irinotecan moiety (as explained above, based on the free base anhydrous) per mole of phospholipid (e.g., a Stability Ratio of 471/0.45 or 1047). The % change in lyso-PC formation was calculated with respect to both Sample 1 and Sample 6 (both of which had pH of 6.5 after manufacture but prior to storage).

***Table 3A:** Irinotecan Liposome Preparation pH and Lyso-PC (after 9 months at 4 °C, 471 g irinotecanmoiety/mol phospholipid, 0.6 M SOS sulfate group concentration)*

Sample	Stability Ratio	Lyso-PC @ 9mos	pH	% lyso-PC @ 9mos relative to Sample 4	% lyso-PC @ 9mos relative to Sample 9
4	785	35.8	6.5	0	+3%
9	785	34.7	6.5	-3%	0
10	785	29	7.25	-19%	-16%
11	785	28.7	7.5	-20%	-17%

***Table 3B**: Irinotecan Liposome Preparation pH and Lyso-PC (after 9 months at 4 °C, 471 g irinotecan moiety/mol phospholipid, 0.45 M SOS trapping agent concentration)*

Sample	Stability Ratio	Lyso-PC @ 9mos	pH	% lyso-PC @ 9mos relative to Sample 1	% lyso-PC @ 9mos relative to Sample 6
1	1047	25.4	6.5	0	+46%
6	1047	17.4	6.5	-31%	0
5	1047	11.1	7.25	-56%	-36%
7	1047	8.1	7.25	-68%	-53%
13	1047	13.8	7.25	-46%	-21%
8	1047	7.1	7.5	-72%	-59%

[00101] In the data in Tables 3A and 3B above, increasing the pH from 6.5 to 7.25 or 7.5 reduced the amount of lyso-PC by about 15-20% for irinotecan SOS liposomes having a Stability Ratio of 785 (Table 3A) and by about 20-70% in irinotecan SOS liposomes having a Stability Ratio of 1047 (Table 3B). This was unexpected in view of prior reports showing that a pH of 6.5 as optimal for minimizing phosphatidylcholine hydrolysis (Grit, M et al, "Hydrolysis of partially saturated egg phosphatidylcholine in aqueous liposome dispersions and the effect of cholesterol incorporation on hydrolysis kinetics," The Journal of pharmacy and pharmacology (1993) v 45, Is 6, pp 490-495).

[00102] FIGS. 4A-4C depict plots showing the mol% of lyso-PC measured after storage of each sample at 4 °C after 0, 1, 3, 6, and/or 9 months in irinotecan liposome preparations having a pH of 7.25 or 7.5, selected from Table 1A and 1B. These plots include a linear regression line for the rate of increase in lyso-PC over time in each sample. A summary of the slope, y-intercept, and R^2 values for each FIG. is shown in Table 4 below. Lower amounts of lyso-PC were observed in irinotecan liposome preparation samples having a Stability Ratio above 942 (e.g., 1047) and pH of 7.25 or 7.5 (e.g., comparing samples 5, 7 and 13 to sample 10 in FIGS. 4A and 4C at pH 7.25, or comparing sample 8 in FIG. 4B to sample 11 in FIG. 4C at pH 7.5). Also, more lyso-PC was measured after 9 months in the irinotecan liposome preparations having a Stability Ratio below 942 (e.g., 785 in Samples 10 and 11, both having more than 20 mol% lyso-PC after 6 months, even at a pH above 6.5).

Table 4: *mol% lyso-PC vs. refrigerated storage time (months) at pH >6.5*

FIG.	Sample	pH	Stability Ratio	y-intercept (mol% lyso-PC)	Slope (mol% lyso-PC per month)	R^2
4A	7	7.25	1047	2.4	0.68	0.9217
4A	5	7.25	1047	0.73	1.1	0.9946
4A, 4B	13	7.25	1047	1.2	1.4	0.9999
4B	8	7.50	1047	1.3	0.65	0.9805
4C	10	7.25	785	8.6	2.4	0.9732
4C	11	7.50	785	8.4	2.3	0.9731

Table 5: *mol% lyso-PC at SR>942 after 6 and 9 months refrigerated storage*

FIG.	Sample	pH	Stability Ratio	[mol% Lyso-PC] at 6 mos.	[mol% Lyso-PC] at 9 mos.
3B	2	6.5	992	17	23.6
3A	1	6.5	1047	19.5	25.4
3A	6	6.5	1047	14.6	17.4
4A	5	7.25	1047	7.1	11.1
4A	7	7.25	1047	7.4	8.1
4B	13	7.25	1047	9.72	13.8
4B	8	7.5	1047	5.4	7.1

Additional Camptothecin Compositions

[00103] Camptothecin compositions can be extended-release compositions comprising one or more camptothecin compound(s) and one or more phospholipid(s) that generate reduced amounts of lyso-phospholipid(s) after periods of refrigerated storage, i.e., 2-8 °C, following manufacturing of the camptothecin composition (e.g., starting when the camptothecin composition is sealed in a sterile container for pharmaceutical administration.

[00104] The stabilized extended release compositions can include a matrix composition comprising a camptothecin compound and phospholipid or other component(s) that can hydrolyze to form lyso-phospholipids. The matrix composition can be configured as a liposome encapsulating the one or more camptothecin compound(s) within a vesicle comprising the phospholipid(s) and other components, such as cholesterol and a lipid covalently linked to PEG.

[00105] In some embodiments of the present invention, the matrix composition is stabilized, for example, by preparing the matrix composition with an amount of an anionic trapping agent and an amount of a camptothecin compound, as well as a specific pH in the medium

containing the matrix composition, effective to reduce the amount of lyso-phospholipid formation in the matrix composition.

[00106] In some embodiments of the present invention, the extended-release composition is a nanoparticle comprising triethylammonium sucrosofate (SOS) and irinotecan releasably-associated with a composition comprising a lipid and/or biocompatible polymer (e.g., a cyclodextrin, biodegradable polymer such as PGA (polyglycolic acid), and/or PLGA (poly(lactic-co-glycolic acid))).

[00107] In other examples, the extended release formulation is a matrix composition comprising a releasably-associated compound such as topotecan, etirinotecan, and/or irinotecan (e.g., nanoparticles or polymers releasably entrapping or retaining the camptothecin or camptothecin derivative compound). The matrix composition can include a biocompatible polymer such as polyethylene glycol (PEG) or functionally equivalent materials. In a preferred embodiment, the biocompatible polymer is polyethylene glycol (MW 2000). In a more preferred embodiment, the biocompatible polymer is methoxy-terminated polyethylene glycol (MW 2000).

[00108] In some embodiments, the extended release formulation can comprise a camptothecin compound conjugated to a biocompatible polymer such as a cyclodextrin or cyclodextrin analog (e.g., sulfated cyclodextrins). For example, the extended release formulation can comprise a cyclodextrin-containing polymer chemically bound to a camptothecin compound (e.g., irinotecan and/or SN-38). A cyclodextrin-camptothecin conjugated compound can be administered at a pharmaceutically acceptable dose. Examples of camptothecin-cyclodextrin conjugate include a cyclodextrin-containing polymer conjugate and related intermediates.

[00109] In some embodiments of the present invention, the extended-release composition comprising a lipid and/or biocompatible polymer comprises a lipid matrix and/or complexing agent(s), such as cyclodextrin-containing compositions formulated to retain the camptothecin compound(s) during storage and then release the compound within the patient's body.

[00110] In some embodiments of the present invention, the matrix composition comprises a phospholipid, such as a phosphatidylcholine derivative, that is stabilized to reduce the formation of lyso-PC during refrigerated storage.

[00111] Preferably, the extended release composition is prepared by a multi-step process comprising the steps of: (a) forming a matrix composition comprising a trapping agent, and (b) contacting the matrix with the camptothecin compound under conditions effective to stably retain the camptothecin compound in a resulting extended-release composition

comprising the trapping agent and the camptothecin compound associated with the matrix composition in a manner permitting the desired release of the camptothecin compound within a subject's body upon administration to the subject.

[00112] In a preferred embodiment, the extended-release composition of the present invention contains irinotecan or a salt thereof in an irinotecan moiety concentration equivalent to that provided by 4.3 mg/mL irinotecan free anhydrous base per mL, while also containing less than about 1 mg/mL (or less than about 20 mol%) lyso-PC at 6 months of refrigerated storage at 4 °C. In a preferred embodiment, the extended-release composition of the present invention contains irinotecan or a salt thereof in an irinotecan moiety concentration equivalent to that provide by 4.3 mg/mL irinotecan free anhydrous base per mL, while also containing less than about 2 mg/mL (or less than about 30 mol%) lyso-PC at 12 months of refrigerated storage 2-8 °C, even more preferably at about 4 °C.

[00113] The extended-release composition can comprise liposomes. Liposomes typically comprise vesicles containing one or more lipid bilayers enclosing an aqueous interior. Liposome compositions usually include liposomes in a medium, such as an aqueous fluid exterior to the liposome. Liposome lipids can include amphiphilic lipid components that, upon contact with aqueous medium, spontaneously form bilayer membranes, such as phospholipids, for example, phosphatidylcholines. Liposomes also can include membrane-rigidifying components, such as sterols, for example, cholesterol. In some cases, liposomes also include lipids conjugated to hydrophilic polymers, such as, polyethyleneglycol (PEG) lipid derivatives that may reduce the tendency of liposomes to aggregate and also have other beneficial effects. One such PEG-lipid is N-(methoxy-PEG)-oxycarbonyl-distearoyl-phosphatidylethanolamine, where PEG moiety has molecular weight of about 2000, or MPEG-2000-DSPE. Liposomes typically have the size in a micron or submicron range and are well recognized for their capacity to carry pharmaceutical substances, including anticancer drugs, such as irinotecan, and to change their pharmaceutical properties in various beneficial ways. Methods of preparing and characterizing pharmaceutical liposome compositions are known in the field (see, e.g., Lasic D. Liposomes: From physics to applications, Elsevier, Amsterdam 1993; G. Gregoriadis (ed.), Liposome Technology, 3rd edition, vol. 1-3, CRC Press, Boca Raton, 2006; Hong et al., US Pat. 8,147,867, incorporated by reference herein in their entirety for all purposes).

[00114] In some embodiments, the liposomes are prepared as described in one or more Examples or other embodiments herein, but the concentration of the final liposome composition is increased so that the formulation contains an irinotecan moiety concentration

equivalent to irinotecan hydrochloride trihydrate at a concentration of about 10, 15, 20, 25, 30, 35, 40, 45, or 50 mg/mL. In some embodiments, the irinotecan moiety concentration is equivalent to irinotecan hydrochloride trihydrate between 5-10, 10-20, 20-30, 30-40 or 40-50 mg/mL. In some embodiments, the liposome compositions mentioned under this section are used to treat brain tumor or any other condition in a mammal, as described U.S. Patent No. 8,658,203, which is incorporated herein by reference in its entirety.

[00115] The formulation of liposomes encapsulating irinotecan can be an injectable formulation containing liposomes (including injectable formulations that can be subsequently diluted with a pharmaceutically acceptable diluent prior to administration to a patient). In some embodiments, the amount of irinotecan or a salt thereof is added to liposomes containing one or more trapping agents, where the irinotecan is present at a concentration of irinotecan moiety equivalent to, in grams of the irinotecan free anhydrous base, 200 g, 300 g, 400 g, 500 g, 600 g, or 700 g per mol phospholipid. In some embodiments, the irinotecan is present during the drug loading process at a concentration of irinotecan moiety equivalent to, in grams of the irinotecan free anhydrous base from 200 to 300 g, from 400 to 550 g, from 450 to 600 g, or from 600 to 700 g per mol phospholipid. Preferably, about 500 g (±10%) moiety loaded into irinotecan liposomes per mol liposome phospholipid, including 471 g irinotecan moiety per mol total irinotecan liposome phospholipid. Specific examples herein include measurements of stabilized irinotecan liposomes containing 471 g irinotecan moiety per mol total liposome phospholipid, as well as irinotecan liposomes containing 500 g irinotecan moiety per mol total liposome phospholipid.

[00116] In some embodiments, the concentration of the irinotecan moiety equivalent to that provided by the irinotecan free anhydrous base in the liposome preparation is about 2.5, about 3.0, about 3.5, about 4.0, about 4.3, about 4.5, about 5.0, about 5.5, or about 6.0 mg/mL. In some embodiments, the concentration of the irinotecan moiety, equivalent to that provided by the irinotecan free anhydrous base in the liposome preparation, is 2.5-3.5, 3.5-4.5, 4.5-5.5, or 5.5-6.5 mg/mL. Most preferably it is 4.5-5.5 mg/mL. In preferred embodiments, the concentration of irinotecan moiety in the liposome preparation is about 4.3 mg/mL irinotecan free base anhydrous per mL, and in a more preferred embodiment, it is 4.3 mg/mL irinotecan free base anhydrous per mL. The liposome preparation can be a vial containing about 43 mg irinotecan free anhydrous base in the liposome preparation having a volume of about 10 mL, which can be subsequently diluted (e.g., into 500 mL of a pharmaceutically acceptable diluent) prior to intravenous administration to a patient.

[00117] Thus some embodiments of the invention provide a method of producing an irinotecan liposome preparation comprising stabilized irinotecan liposomes encapsulating irinotecan sucrose octasulfate (SOS) in an unilamellar lipid bilayer vesicle consisting of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE), comprising the steps of: (a) contacting a solution containing irinotecan with a trapping agent liposome encapsulating a triethylammonium (TEA) cation, and sucrose octasulfate (SOS) trapping agent at a sulfate concentration of 0.4-0.5 M (provided from TEA_8SOS) without irinotecan under conditions effective to load 500 g (±10%) of the irinotecan moiety per mol phospholipid into the trapping agent liposome to form the irinotecan SOS liposomes, and (b) combining the irinotecan SOS liposomes with 2-[4-(2-hydroxyethyl) piperazin-1-yl]ethanesulfonic acid (HEPES) to obtain an irinotecan liposome preparation having a pH of 7.25-7.50, to obtain an irinotecan liposome preparation stabilized to form less than 10 mol% lyso-phosphatidylcholine (lyso-PC) (with respect to the total amount of phosphatidylcholine in the irinotecan liposomes) during 3 months of storage at 4 °C.

[00118] Storage stabilized irinotecan liposomes can be prepared in multiple steps comprising the formation of a TEA containing liposome, followed by loading of irinotecan into the liposome as the TEA leaves the liposome. The first step can include forming the TEA-sucrosofate containing liposome by hydrating and dispersing the liposome lipids in the solution of TEA sucrosofate. This can be performed, for example, by dissolving the lipids, including DSPC and cholesterol, in heated ethanol, and dispersing the dissolved and heated lipid solution in the TEA-sucrosofate aqueous solution at the temperature above the transition temperature (T_m) of the liposome lipid, e.g., 60 °C or greater. The lipid dispersion can be formed into liposomes having the average size of 75-125 nm (such as 80-120 nm, or in some embodiments, 90-115 nm), by extrusion through track-etched polycarbonate membranes with the defined pose size, e.g., 100 nm. The TEA-sucrosofate can include at least 8 molar equivalents of TEA to each molar equivalent of sucrosofate to obtain a solution that can have a sulfate concentration of about 0.40-0.50 M, and a pH (e.g., about 6.5) that is selected to prevent unacceptable degradation of the liposome phospholipid during the dispersion and extrusion steps (e.g., a pH selected to minimize the degradation of the liposome phospholipid during these steps). Then, the non-entrapped TEA-SOS can be removed from the liposome dispersion, e.g., by dialysis, gel chromatography, ion exchange or ultrafiltration prior to irinotecan encapsulation. These liposomes can be stabilized by loading enough irinotecan into the liposomes to reduce the amount of TEA in the resulting liposome composition to a level

that results in less than a given maximum level of lyso-PC formation after 180 days at 4 °C, or, more commonly, at 5 ± 3 °C, measured, e.g., in mg/mL/month, or % PC conversion into a lyso-PC over a unit time, such as, mol% lyso-PC/ month. Next, the TEA exchanged from the liposomes into the external medium during the loading process, along with any unentrapped irinotecan, is typically removed from the liposomes by any suitable known process(es) (e.g., by gel chromatography, dialysis, diafiltration, ion exchange or ultrafiltration). The liposome external medium can be exchanged for an injectable isotonic fluid (e.g. isotonic solution of sodium chloride), buffered at a desired pH.

[00119] In some embodiments, irinotecan liposome compositions containing about 3.9-4.7 mg/mL of irinotecan and less than 20% lyso-PC after 180 days at 4 °C can be obtained when the amount of TEA is less than about 25 ppm, or less about 20 ppm. Raising the pH of the irinotecan liposome composition outside the liposome can also storage stabilize the irinotecan sucrosofate liposomes containing more than 25 ppm TEA, resulting in irinotecan liposomes having less than 20% additional lyso-PC formation after 180 days at 4 °C. For example, irinotecan liposome compositions containing about 4-5 mg irinotecan/mL and 100 ppm of TEA and having a pH of about 7-8 outside the liposome can also have less than 20% lyso-PC formation after 180 days at 4 °C. In another example, liposome compositions containing about 3.9-4.7 mg/mL irinotecan and a pH of the liposome outer medium in the range of 7-8, with the amount of residual TEA of less than about 25 ppm (or preferably, less than 20 ppm), the amount of lyso-PC accumulated in the liposome composition over 180 days at 4 degree C can be 10 mol.% or less.

[00120] The invention thus provides an irinotecan liposome composition comprising irinotecan sucrosofate encapsulated in a phospholipid liposome having a Lyso-PC Stability Ratio of at least 990 (e.g., 990-1100, or about 1111)

[00121] The invention also provides an irinotecan liposome composition, the composition comprising 4.3 mg/mL(±10%) moiety equivalent to that provided by irinotecan free anhydrous base and 0.4-0.5 M concentration of sulfate encapsulated in a vesicle comprising DSPC and cholesterol in a 3:2 molar ratio, and a ratio of 400-600 g irinotecan /mol phospholipid in the vesicle.

[00122] The invention also provides irinotecan liposome composition comprising a total of about 4.3 mg irinotecan moiety/mL, with at least 98% of the irinotecan being encapsulated with sucrose octasulfate (SOS) at a irinotecan:SOS mole ratio of about 8:1 within a liposome composition, the liposomes having an average size of 75-125 nm. The size of the stabilized high-density irinotecan liposomes is preferably about 110 nm (±20 nm), and more preferably

110 nm (±10 nm) (measured after liposomal drug loading). Preferably, at least about 95% of the irinotecan in the pharmaceutical composition is encapsulated within the liposome. The liposome preferably comprises DSPC and cholesterol in a 3:2 molar ratio.

[00123] The invention can also provide a method of producing a pharmaceutical comprising stabilized irinotecan liposomes encapsulating irinotecan sucrose octasulfate (SOS) in an unilamellar lipid bilayer vesicle consisting of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE), comprising the steps of: (a) contacting irinotecan with a trapping agent liposome encapsulating a triethylammonium (TEA) cation, and sucrose octasulfate (SOS) trapping agent at a sulfate concentration of 0.4-0.5 M, as TEA_8SOS without irinotecan under conditions effective to load the irinotecan moiety into the trapping agent liposome and permit the release of the TEA cation from the trapping agent liposome, to form the irinotecan SOS liposomes, (b) combining the irinotecan SOS liposomes with 2-[4-(2-hydroxyethyl) piperazin-1-yl]ethanesulfonic acid (HEPES) to obtain an irinotecan liposome preparation having a pH of 7.25-7.50, to obtain an irinotecan liposome preparation stabilized to form less than 10 mol% lyso-phosphatidylcholine (lyso-PC) (with respect to the total amount of phosphatidylcholine in the irinotecan liposomes) during 3 months of storage at 4 °C, and (c) formulating the combination of irinotecan SOS liposomes and HEPES as a pharmaceutical.

[00124] In some embodiments of these methods, the irinotecan SOS liposomes in the irinotecan liposome preparation contain a total of less than 100 ppm TEA. In some embodiments, the unilamellar lipid bilayer vesicle consists of 6.81 mg/mL 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 2.22 mg/mL cholesterol, and 0.12 mg/mL methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE). In some embodiments, the irinotecan liposome preparation comprises a total of 500 g (±10%) irinotecan per mol of total stabilized irinotecan liposome phospholipid, and at least 98% of the irinotecan in the irinotecan liposome preparation is encapsulated within the irinotecan liposomes. In some embodiments, the irinotecan liposome preparation further comprises 4.05 mg/mL 2-[4-(2-hydroxyethyl) piperazin-1-yl]ethanesulfonic acid (HEPES). In some embodiments, the irinotecan liposome preparation further comprises 8.42 mg/mL sodium chloride. In some embodiments, the irinotecan liposome preparation, has an irinotecan moiety concentration equivalent to that provided by about 4.3 mg/mL irinotecan free anhydrous base. In some embodiments, the stabilized irinotecan liposomes encapsulate irinotecan and SOS in a compound of formula (I), where x=8.

[00125] In some embodiments, the composition contains less than 2 mol% lyso-PC after 3 months of storage at 2-8 °C. In some embodiments, the composition contains less than 5 mol% lyso-PC after 3 months of storage at 2-8 °C. In some embodiments, the liposomal composition contains less than 10 mol% lyso-PC after 6 months of storage at 2-8 °C. In some embodiments, the composition contains less than 10 mol% lyso-PC after 9 months storage at 2-8 °C. In some embodiments, the composition contains less than 5 mol% lyso-PC after 6 months of storage at 2-8 °C. In some embodiments, the composition contains less than 5 mol% lyso-PC after 9 months storage at 2-8 °C. In some embodiments, the composition contains less than 2 mol% lyso-PC after 6 months of storage at 2-8 °C. In some embodiments, the composition contains than 2 mol% lyso-PC after 9 months storage at 2-8 °C. In some embodiments, the composition contains less than 10 mol% lyso-PC after 12 months storage at 2-8 °C. In some embodiments, the composition contains less than 5 mol% lyso-PC after 12 months storage at 2-8 °C. In some embodiments, the composition contains less than 2 mol% lyso-PC after 12 months storage at 2-8 °C. In some embodiments, the composition containing less than 10 mol% lyso-PC after 24 months storage at 2-8 °C. In some embodiments, the composition contains less than 5 mol% lyso-PC after 24 months storage at 2-8 °C. In some embodiments, the composition contains less than 2 mol% lyso-PC after 24 months storage at 2-8 °C. In some embodiments, the composition contains less than 100 ppm of a substituted ammonium. In some embodiments, the composition contains between 20 and 80 ppm of a substituted ammonium compound, which is protonated TEA or DEA.

[00126] In other embodiments, the stabilized camptothecin composition is provided as a kit comprising one or more component vials for the preparation of the camptothecin composition. For example, a kit for the preparation of liposomal irinotecan can include the following (stored in separate containers or separate portions of the same container:

- an irinotecan solution (e.g., irinotecan HCl for injection);
- a liposome encapsulating a trapping agent (e.g., trapping agent liposomes formed from a sucrose octasulfate solution); and
- instructions for combining the irinotecan solution and the trapping agent liposomes to form a liposomal irinotecan composition comprising a therapeutically effective amount of irinotecan encapsulated in liposomal irinotecan liposomes (e.g., 500 g (±10%) irinotecan per mol total phospholipid in the trapping agent liposomes, and 4.3 mg total irinotecan per mL of liposomal irinotecan composition).

Therapeutic Use of Camptothecin Compositions

[00127] The camptothecin compositions - including irinotecan liposomes and other compositions and preparations disclosed herein of the invention can be used in therapy and methods of treatment, and or in the preparation of medicaments for the treatment of disease, such as cancer. In some embodiments, a therapy comprises administration of a camptothecini composition for the treatment of cancer. For example the cancer is selected from the group consisting of basal cell cancer, medulloblastoma cancer, liver cancer, rhabdomyosarcoma, lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, chronic or acute leukemia, lymphocytic lymphomas, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system, primary central nervous system lymphoma, spinal axis tumors, brain stem glioma and pituitary adenoma, or a combination of one or more of these cancers. In some embodiments the cancer is pancreatic cancer, optionally adenocarcinoma of the pancreas, such as metastatic adenocarcinoma of the pancreas, for example where disease progression has occurred following gemcitabine-based therapy. In some embodiments the cancer is ovarian cancer. In some embodiments the cancer is small cell lung cancer. In some embodiments, the cancer is biliary tract cancer.

[00128] When used as a therapy, the liposome composition may be used in a treatment regimen with one or more other compounds or compositions. The administration of the liposome composition with one or more other compounds or compositions may be simultaneous, separate or sequential. The one or more other compounds or compositions may be further therapeutics, e.g. further anticancer agents, or may be compounds which are designed to ameliorate the negative side effects of the therapeutic agents. In some embodiments, the liposome composition is administered with leucovorin. In some embodiments, the liposome composition is administered with 5-fluorouracil (5-FU). In some embodiments, the liposome composition is administered with leucovorin and 5-fluorouracil (5-FU). This three-way regimen can be used to treat pancreatic cancer, as discussed in the

previous paragraph. 5-FU can be administered at a dose of 2400 mg/m^2, and leucovorin can be administered at a dose of 200 mg/m2 (l form) or 400 mg/m^2 (l + d racemic form). In some embodiments, the composition is also administered in a treatment regimen with gemcitabine.

[00129] In some embodiments where the liposome composition is used to treat ovarian cancer, the liposome composition is administered with a PARP (poly ADP ribose polymerase) inhibitor.

[00130] In some embodiments, the extended release matrix can be a nanoparticle (e.g. silica or polymer) or a polymer aggregate (e.g., PEG polymer) configured to retain the trapping agent. During drug loading, the matrix can be contacted with the camptothecin compound under conditions effective to retain both the camptothecin compound and the trapping agent, forming the stable extended release formulation.

[00131] In some embodiments, stabilized camptothecin composition is an irinotecan SOS liposome preparation is formulated for intraparenchymal administration to a patient during a convection enhanced delivery therapy. The concentration of the irinotecan moiety, equivalent to that provided by the irinotecan free anhydrous base in the final liposome preparation is about 17, about 20, about 25, about 30, about 35, or about 40 mg/mL. In some embodiments, the concentration of the irinotecan moiety, equivalent to that provided by the irinotecan free anhydrous base in the final liposome preparation is 17-20, 17-25, 17-30, 17-35, or 17-40 mg/mL. Most preferably, the total concentration of the irinotecan moiety, equivalent to that provided by the irinotecan free anhydrous base (e.g., as irinotecan sucrose octasulfate) in the irinotecan liposome preparation is 17 mg/mL, or 35 mg/mL. The liposome preparation can be in a sterile container enclosing irinotecan sucrose octasulfate liposomes in the liposome preparation at an irinotecan moiety concentration equivalent to that provided by about 17 mg/mL or about 35 mg/mL or 17-35 mg/ml irinotecan free anhydrous base for local administration to a patient (e.g., into the brain of a patient diagnosed with a glioma, to a location within the brain as part of a convection enhanced delivery therapy). The 17-35 mg/mL concentration of irinotecan liposomes can be equivalently expressed as the amount of irinotecan free anhydrous base present in 20-40 mg of irinotecan hydrochloride trihydrate, per mL of the irinotecan liposome preparation. For example, the liposomal irinotecan preparation can be administered into the brain of a patient (e.g., via one or more catheters surgically placed in an intra-tumoral location) at doses providing a total of irinotecan moiety equivalent to that provided by 17 mg, 26 mg, 52 mg, or 70 mg total irinotecan free anhydrous base. The irinotecan total volume of the irinotecan liposome preparation delivered into the

intra-tumoral location within the brain of the patient can be about 1-2 mL (e.g., 1.0, 1.5, or 2.0 mL) over a period of about 2-4 hours (e.g., 2-3 hours, 3-4 hours, or 2-4 hours).

[00132] The irinotecan liposomes preferably contain irinotecan sucrosofate encapsulated within a vesicle formed from lipids comprising DSPC and cholesterol in a 3:2 molar ratio. The vesicle can also contain a polyethylene-glycol (PEG) derivatized phospholipid, such as MPEG-2000-DSPE. The amount of MPEG-2000-DSPE can be less than 1 mol% of the liposome lipid (e.g, about 0.3 mol.% in a vesicle consisting of DSPC, cholesterol and MPEG-2000-DSPE in a 3:2:0.015 molar ratio). The PEG can be distributed on both the inside and the outside of the liposome lipid vesicle enclosing the irinotecan. The encapsulated irinotecan is preferably in the form of a salt with sulfate ester of sucrose (sucrosofate), such as irinotecan sucrosofate (CAS Registry Number 1361317-83-0). Preferably, at least 95% and most preferably at least about 98% of the irinotecan in the irinotecan liposome composition is encapsulated within a liposome vesicle, with a total irinotecan moiety concentration of about 3.87-4.73 mg irinotecan (free anhydrous base) per mL of the irinotecan liposome composition. The pH of the irinotecan liposome composition is preferably about 6.5-8.0 outside the liposome, or about 6.6-8.0, 6.7-8.0, 6.8-8.0, 6.9-8.0, or 7.0-8.0, and preferably about 7.2-7.6. In some embodiments, the pH is about 7.2-7.5. In some embodiments, the pH is about 7.25. In other embodiments, the pH is about 7.25-7.5. In other embodiments, the pH is about 7.4-7.5.

Combination Embodiments

[00133] The features from the numbered embodiments herein can be combined with features from other embodiments disclosed here, including both embodiments referring to compositions and embodiments referring to preparations.

[00134] The methods set out above share features in common with the embodiments of the compositions and preparations set out elsewhere in the specification because they relate to the production of these compositions and preparations. Features disclosed in respect of the compositions and preparations may also be combined with the methods disclosed in the preceding paragraph. Accordingly, the features of the preceding subsections, and elsewhere herein, such as in the numbered embodiments section below, can be combined with the features disclosed in the methods in the paragraphs of this subsection.

[00135] For example, the following are examples of various combinations of embodiments disclosed and/or exemplified herein:

- An irinotecan liposome composition that, after storage for 180 days at 4 degrees C,

contains about 3.9-4.7 mg/ml of irinotecan moiety and less than 20% lyso-PC.

- An irinotecan liposome composition comprising irinotecan sucrosofate encapsulated in a phospholipid liposome having a lyso-PC Stability Ratio of at least 990 (e.g., 990-1100, or about 1111).
- An irinotecan liposome composition, the composition comprising 4.3 mg/mL(±10%) irinotecan moiety and 0.4-0.5 M concentration of sulfate encapsulated in a vesicle comprising DSPC and cholesterol in a 3:2 molar ratio, and a ratio of 450-550 g irinotecan /mol total phospholipid in the vesicle.
- An irinotecan liposome composition comprising a total of about 4.3 mg irinotecan moiety/mL, with at least 98% of the irinotecan being encapsulated with sucrose octasulfate (SOS) at a irinotecan:SOS mole ratio of about 8:1 within a liposome composition, the liposomes having an average size of 75-125 nm.
- The composition of any preceding embodiment, wherein the irinotecan liposome is obtained by a process comprising the step of contacting irinotecan with triethylammonium (TEA) sucrosofate encapsulated within the phospholipid liposome.
- The composition of the preceding embodiment, wherein the concentration of TEA-SOS is about 0.40-0.50 M.
- The composition of any of the preceding embodiments, wherein the size of the liposome is about 110 nm (±10%).
- The composition of any of the preceding embodiments, comprising about 433 g irinotecan moiety/mol phospholipid.
- The composition of any of the preceding embodiments, wherein the irinotecan liposome composition contains less than about 100 ppm of triethylamine.
- The composition of any of the preceding embodiments, wherein the irinotecan liposome composition is a solution of liposomes in a liquid, wherein the liquid outside of the irinotecan liposomes has a pH of about 7.0-8.0, for example 7.25-7.5, such as 7.25, optionally wherein the liquid outside of the irinotecan liposomes is a pharmaceutically acceptable injectable fluid.
- The composition of any preceding embodiments, comprising irinotecan moiety in the amount equivalent to that provided by 4.5-5.5 mg/ml irinotecan hydrochloride trihydrate.
- The composition of any preceding embodiments, wherein at least about 95% of the irinotecan in the irinotecan liposome composition is encapsulated within the liposome.
- The composition of any of the preceding embodiments, wherein the liposome comprises

DSPC and cholesterol in a 3:2 molar ratio, such as wherein the liposome comprises DSPC, cholesterol, and MPEG(2000)-DSPE at the molar ratio of 3:2:0.015.

- The composition of any of the preceding embodiments, having a Stability Ratio of 990-1200.
- The composition of any of the preceding embodiments having liposomally encapsulated irinotecan/sucrosofate gram-equivalent ratio of at least 0.9, at least 0.95, at least 0.98, at least 0.99 or essentially 1.0.
- The composition of any of the preceding embodiments wherein liposome phospholipid contains no more than 20 mol% lyso-PC after storage for 180 days at about 4 degrees C.
- The composition of any of the preceding embodiments wherein the irinotecan liposome composition further comprises a pharmaceutically acceptable injectable fluid having a pH of about 7.0-8.0 outside the irinotecan liposome, comprises 4.3 mg/mL irinotecan calculated as a free base, and is optionally obtained by a process comprising the step of contacting irinotecan with triethylammonium (TEA) sucrosofate encapsulated within the phospholipid liposome, optionally having a concentration of encapsulated TEA sucrosofate of about 0.40-0.50N.
- The composition of any preceding embodiments wherein the composition comprises about 433 g irinotecan moiety/mol phospholipid, and not more than about 100 ppm of triethylammonium encapsulated within the phospholipid liposome.
- The composition of any preceding embodiments which has an encapsulated irinotecan/sucrosofate gram-equivalent ratio of at least 0.9.
- The composition of any preceding embodiments in which at least 90%, such as at least 92%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% (in other words, essentially all) of the encapsulated irinotecan sucrosofate is in the precipitated or gelated form of a stoichiometric salt comprising eight molecules of irinotecan per one molecule of sucrosofate.
- The composition of any preceding embodiments, in which at least 98%, such as at least 99%, of the encapsulated irinotecan sucrosofate is in the precipitated or gelated form of a stoichiometric salt comprising eight molecules of irinotecan per one molecule of sucrosofate.
- The irinotecan liposome composition of any preceding embodiment having no more than about 100 ppm of triethylammonium (TEA).

- The irinotecan liposome composition of any preceding embodiment, having no more than about 20 ppm of triethylammonium (TEA).
- The irinotecan liposome composition of any preceding embodiment having a total volume of about 10 mL.
- The irinotecan liposome composition of any preceding embodiment, comprising 6.81 mg/mL 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 2.22 mg/mL cholesterol, and 0.12 mg/mL methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE).
- The irinotecan liposome composition of any preceding embodiment, comprising polyethylene glycol both inside and the outside of the irinotecan liposome.
- A stabilized injectable unit dose irinotecan liposome composition formulated for administration to a patient, the composition comprising a dose of irinotecan sufficient to deliver 70 mg irinotecan per m^2 of the patient body surface area, wherein:
    - at least 99% of the irinotecan is encapsulated in a vesicle comprising phospholipid and cholesterol and wherein up to 20 mol.% of the phospholipid is lyso-PC, the balance being DSPC, wherein the vesicle is in an injectable fluid having the pH in the range of 7.0-8.0; or
    - the injectable unit dose liposome composition is a unit dose of the liposome compositions of any one of the embodiments above.
- An injectable irinotecan liposome unit dosage form comprising:
    - at least about 98% of the irinotecan in the unit dosage form encapsulated in a liposome comprising phospholipid, said phospholipid containing not more than about 20 mol.% lyso-PC; and
    - a liposome composition according to any one of the embodiments above.
- The unit dosage form disclosed in an embodiment above, wherein the irinotecan is encapsulated in a vesicle enclosed by a lipid membrane consisting essentially of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE).
- The unit dosage form of embodiment 29 or 30, wherein the unit dosage form comprises at least about 6.81 mg/mL 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), about 2.22 mg/mL cholesterol, and about 0.12 mg/mL methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE) L.

- The unit dosage form of any of the embodiments 29-31, wherein the unit dosage form further comprises 2-[4-(2-hydroxyethyl) piperazin-1-yl]ethanesulfonic acid (HEPES) as a buffer and sodium chloride as an isotonicity reagent.
- The liposome composition according to any one of embodiments 1-27, or unit dose according to any one of embodiments 29-32, for use in therapy.
- The liposome composition or unit dose disclosed in an embodiment herein for use in treating cancer.
- The liposome composition or unit dose for use disclosed in an embodiment herein, wherein the cancer is selected from the group consisting of basal cell cancer, medulloblastoma cancer, liver cancer, rhabdomyosarcoma, lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, chronic or acute leukemia, lymphocytic lymphomas, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system, primary central nervous system lymphoma, spinal axis tumors, brain stem glioma and pituitary adenoma, or a combination of one or more of these cancers.
- The liposome composition or unit dose according to any above embodiment, wherein the cancer is pancreatic cancer, optionally adenocarcinoma of the pancreas, such as metastatic adenocarcinoma of the pancreas, for example where disease progression has occurred following gemcitabine-based therapy.
- The liposome composition or unit dose according to any above embodiment, wherein the cancer is colon cancer.
- The liposome composition or unit dose according to any above embodiment, wherein the liposome composition or unit dose is for use with leucovorin and/or 5-flurouracil, optionally wherein administration of liposome composition or unit dose, leucovorin and/or 5-flurouracil is simultaneous, separate or sequential.

- The liposome composition or unit dose according to any one of embodiments above, wherein the liposome is administered in a dose to provide an amount of irinotecan equivalent to 80 mg/m^2 of irinotecan hydrochloride trihydrate.
- A method of treating metastatic adenocarcinoma of the pancreas after disease progression following gemcitabine-based therapy in patient in need thereof, comprising intravenously administering to the patient an injectable irinotecan liposome unit dosage form of any of the embodiments herein or the unit dose according to any embodiments above, comprising at least about 98% of the irinotecan in the unit dosage form encapsulated in a liposome comprising phospholipid containing less than about 20% lyso-PC in an amount providing an amount of irinotecan equivalent to 80 mg/m^2 of irinotecan hydrochloride trihydrate
- A storage stabilized liposomal irinotecan composition having a pH of 7.00-7.50 and comprising a dispersion of irinotecan liposomes encapsulating irinotecan sucrose octasulfate in unilamellar bilayer vesicles consisting of cholesterol and the phospholipids 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE), at a concentration of irinotecan moiety equivalent to, in g of irinotecan free anhydrous base, 500 mg irinotecan per mmol total liposome phospholipid and 4.3 mg irinotecan per mL of the liposomal irinotecan composition, the storage stabilized liposomal irinotecan composition stabilized to form less than 1 mg/mL Lyso-PC during 6 months of storage at 4 °C.
- The liposomal irinotecan composition of an embodiment above, made by a process comprising steps of:
  (a) forming a lipid dispersion in a solution made from DEA_8SOS having a sulfate concentration of from 0.4 to 0.5 M and a pH between from 5 to 7, the lipids in said dispersion being DSPC, cholesterol and MPEG-2000-DSPE in an about 3:2:0.015, respectively, mole ratio;
  (b) extruding the lipid dispersion between 60-70 °C through at least one 0.1 μm membrane to form liposomes;
  (c) substantially removing ions derived from DEA_8SOS and/or DEA_8SOS that are outside the liposomes;

(d) contacting the liposomes at a temperature between 60-70 °C with a solution made using irinotecan free base or irinotecan salt, thereby forming a preparation of liposomes encapsulating irinotecan;

(e) substantially removing substances derived from the TEA_8SOS and/or DEA_8SOS and irinotcan ingredients that are outside the liposomes; and

(f) adjusting the pH of the composition to be from 7.0 to 7.5.

- The liposomal irinotecan composition of any embodiment above, wherein the lipid dispersion is extruded through at least two stacked 0.1 μm polycarbonate membranes.
- The liposomal irinotecan composition of any embodiment above, where the liposomes have a mean size of 110 nm as determined by dynamic light scattering and where the size is determined by the method of cumulants.
- The liposomal irinotecan composition of any embodiment above, having a total irinotecan moiety content equivalent to of 4.3 mg/ml irinotecan free base anhydrous.
- The liposomal irinotecan composition of any embodiment above, wherein:

  in step (a) the liposomes are formed from DEA_8SOS having a sulfate concentration of between 0.43-0.47 M; and

  in step (d) the solution made using irinotecan free base or an irinotecan salt has an irinotecan moiety content equivalent to 500 g (±10%) of irinotecan free base anhydrous per mole of DSPC; and

  in step (f) adjusting the pH of the composition to be from 7.2 to 7.3.
- The liposomal composition of any one of the previous embodiments, containing less than 1 mol% lyso-phosphatidylcholine (lyso-PC) prior to storage at about 4 °C, and 20 mol% or less (with respect to total liposome phospholipid) of lyso-PC after 180 days of storage at about 4 °C.
- The liposomal composition of any embodiment above, containing 20 mol% or less (with respect to total liposome phospholipid) of lyso-phosphatidylcholine (lyso-PC) after 6, 9 or 12 months of storage at about 4 °C.
- The liposomal irinotecan composition of any embodiment above, comprising a total of 6.1 to 7.5 mg DSPC/ml, 2 to 2.4 mg cholesterol /ml, and 0.11 to 0.13 mg MPEG-2000-DSPE/ml, all in an aqueous isotonic buffer.
- The liposomal irinotecan composition of any embodiment above, wherein the liposomal irinotecan comprises the irinotecan liposomes in an isotonic HEPES aqueous buffer at a concentration of between 2 and 20 mM.

- The liposomal irinotecan composition of any embodiment above, further comprising sodium chloride at a concentration of from 130-160 mM.
- The liposomal irinotecan composition of any embodiment above, wherein the irinotecan encapsulated in the liposomes is in a gelated or precipitated state as a sucrose octasulfate salt.
- The liposomal irinotecan composition of any embodiment above, wherein the irinotecan liposomes have a diameter of 95-115 nm, as measured by quasi-elastic light scattering.
- The liposomal irinotecan composition of any embodiment above, comprising a total of 6.81 mg DSPC/ml, 2.22 mg cholesterol /ml, and 0.12 mg MPEG-2000-DSPE/ml, 4.05 mg/mL HEPES aqueous buffer and 8.42 mg sodium chloride/mL.
- The liposomal irinotecan composition of any embodiment above, having a pH of 7.25, wherein the irinotecan liposomes have a diameter of 110 nm as measured by quasi-elastic light scattering.
- The liposomal irinotecan composition of any embodiment above, forming less than 1 mg/mL lyso-phosphatidylcholine (lyso-PC) after 6 months of storage at about 4 °C.
- The liposomal irinotecan composition of any embodiment above, made by a process comprising steps of:

  (a) forming a lipid dispersion in a solution of DEA_8SOS having a sulfate concentration of about 0.45 M and a pH of about 6.5, the lipids in said dispersion consisting of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE) in a mole ratio of 3:2:0.015, respectively;

  (b) extruding the lipid dispersion between 60-70 °C through at least one 0.1 μm membrane to form liposomes;

  (c) removing ions derived from DEA_8SOS that are outside the liposomes;

  (d) contacting the liposomes at a temperature between 60-70 °C with a solution made using irinotecan hydrochloride trihydrate, to form a preparation of liposomes encapsulating about 500 g (±10%) irinotecan per mol total liposome phospholipid;

  (e) removing substances derived from the TEA_8SOS and irinotcan ingredients that are outside the liposomes; and

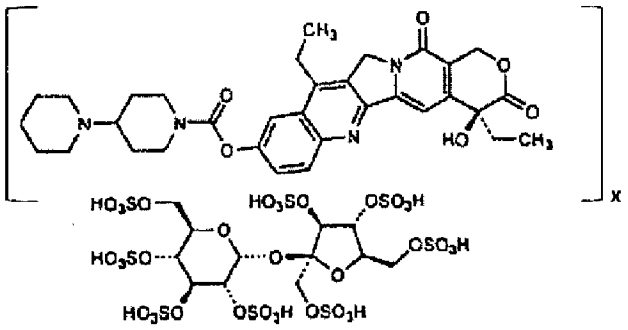
  (f) adjusting the pH of the composition to be about 7.3.
- The liposomal irinotecan composition of any embodiment above, comprising a total of less than 100 ppm of DEA.

- The liposomal irinotecan composition of any embodiment above, comprising a total of less than 100 ppm of DEA.
- The liposomal irinotecan composition of any embodiment above, wherein at least 98% of the irinotecan is encapsulated in the irinotecan liposomes after 6 months of storage at about 4 °C.
- An irinotecan liposome preparation comprising stabilized irinotecan liposomes encapsulating irinotecan sucrose octasulfate (SOS) in an unilamellar lipid bilayer vesicle approximately 110 nm in diameter consisting of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE), wherein the stabilized irinotecan liposomes are obtained by a process comprising the steps of:
  (a) contacting irinotecan with a trapping agent liposome encapsulating a diethylammonium (DEA) cation and sucrose octasulfate (SOS) trapping agent at a concentration of 0.4-0.5 M (based on sulfate group concentration) as TEA_8SOS without irinotecan under conditions effective to load 500 g (±10%) of the irinotecan moiety per mol total liposome phospholipid into the trapping agent liposome and permit the release of the DEA cation from the trapping agent liposome, to form the irinotecan SOS liposomes, and
  (b) combining the irinotecan SOS liposomes with 2-[4-(2-hydroxyethyl) piperazin-1-yl]ethanesulfonic acid (HEPES) to obtain an irinotecan liposome preparation having a pH of 7.25-7.50, to obtain an irinotecan liposome preparation stabilized to form less than 10 mol% lyso-phosphatidylcholine (lyso-PC) (with respect to the total amount of phosphatidylcholine in the irinotecan liposomes) during 3 months of storage at 4 °C.
- The irinotecan liposome preparation any embodiment above, wherein the irinotecan SOS liposomes in the irinotecan liposome preparation contain a total of less than 100 ppm TEA.
- The irinotecan liposome preparation of any embodiment above wherein the unilamellar lipid bilayer vesicle consists of 6.81 mg/mL 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 2.22 mg/mL cholesterol, and 0.12 mg/mL methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE).
- The irinotecan liposome preparation of any embodiment above, comprising a total of 500 mg irinotecan per mol of total stabilized irinotecan liposome phospholipid, and at least 98% of the irinotecan in the irinotecan liposome preparation is encapsulated within the

irinotecan liposomes.

- The irinotecan liposome preparation of any embodiment above, wherein the irinotecan liposome preparation further comprises about 4.05 mg/mL 2-[4-(2-hydroxyethyl) piperazin-1-yl]ethanesulfonic acid (HEPES) at a pH of about 7.25-7.50.
- The irinotecan liposome preparation of any embodiment above, wherein the irinotecan liposome preparation further comprises about 8.42 mg/mL sodium chloride.
- The irinotecan liposome preparation of any embodiment above, having a total of about 4.3 mg irinotecan per mL of the irinotecan liposome preparation.
- The composition of any preceding embodiment, wherein the irinotecan liposome is obtained by a process comprising the step of contacting irinotecan with ammonium encapsulated within the phospholipid liposome.
- An irinotecan liposome preparation comprising stabilized irinotecan liposomes encapsulating irinotecan sucrose octasulfate (SOS) in an unilamellar lipid bilayer vesicle approximately 110 nm in diameter consisting of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE), wherein the stabilized irinotecan liposomes are obtained by a process comprising the steps of:
  (a) contacting irinotecan with a trapping agent liposome encapsulating a ammonium cation and sucrose octasulfate (SOS) trapping agent under conditions effective to load 500 g (±10%) of the irinotecan moiety per mol total liposome phospholipid into the trapping agent liposome and permit the release of the ammonium cation from the trapping agent liposome, to form the irinotecan SOS liposomes, and
  (b) combining the irinotecan SOS liposomes with 2-[4-(2-hydroxyethyl) piperazin-1-yl]ethanesulfonic acid (HEPES) to obtain an irinotecan liposome preparation having a pH of 7.25-7.50, to obtain an irinotecan liposome preparation stabilized to form less than 10 mol% lyso-phosphatidylcholine (lyso-PC) (with respect to the total amount of phosphatidylcholine in the irinotecan liposomes) during 3 months of storage at 4 °C.
- An SN38 liposome preparation comprising stabilized liposomes comprising irinotecan and/or SN-38 in a liposome comprising 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE), stabilized to form less than 10 mol% lyso-phosphatidylcholine (lyso-PC) (with respect to the total amount of phosphatidylcholine in the liposomes) during 3 months of storage at 4 °C.

- The irinotecan liposome preparation of any embodiment above, wherein the stabilized irinotecan liposomes encapsulate 30-100 ppm TEA or DEA, irinotecan and SOS in a compound of formula (I), where x is 8.



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[00136] In one embodiment, the irinotecan liposome composition disclosed herein is a stabilized irinotecan liposome composition comprising irinotecan sucrosofate encapsulated in a phospholipid liposome having a Lyso-PC Stability Ratio of at least 990 (e.g., 990-1100, or about 1111), wherein the liposome composition comprises at least one of the following features:

(i) the size of the liposome is about 110 nm (±10%),

(ii) the composition comprises about 433 g or at least about 433 g irinotecan moiety/mol phospholipid

(iii)the composition contains less than about 100 ppm of triethylamine,

(iv)the composition comprises a pharmaceutically acceptable injectable fluid having a pH of about 7.25 outside the irinotecan liposome,

(v) the liposomes comprise DSPC and cholesterol in a 3:2 molar ratio

(vi)the composition has a liposomally encapsulated irinotecan/sucrosofate gram-equivalent ratio of at least 0.9, at least 0.95, at least 0.98, or essentially 1.0; and

(vii) at least 90%, such as at least 92%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% (in other words, essentially all) of the encapsulated irinotecan sucrosofate is in the precipitated or gelated form of a stoichiometric salt comprising eight molecules of irinotecan per one molecule of sucrosofate.

[00137] In one embodiment, the irinotecan liposome composition disclosed herein is a stabilized irinotecan liposome composition comprising irinotecan sucrosofate encapsulated in

a phospholipid liposome having a Lyso-PC Stability Ratio of at least 990 (e.g., 990-1100, or about 1111), wherein:

(i) the size of the liposome is about 110 nm (±10%),

(ii) the composition comprises about 433 g or at least about 433 g irinotecan moiety/mol phospholipid

(iii)the composition contains less than about 100 ppm of triethylamine,

(iv)the composition comprises a pharmaceutically acceptable injectable fluid having a pH of about 7.25 outside the irinotecan liposome,

(v) the liposomes comprise DSPC and cholesterol in a 3:2 molar ratio

(vi)the composition has a liposomally encapsulated irinotecan/sucrosofate gram-equivalent ratio of at least 0.9, at least 0.95, at least 0.98, or essentially 1.0; and

(vii) at least 90%, such as at least 92%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% (in other words, essentially all) of the encapsulated irinotecan sucrosofate is in the precipitated or gelated form of a stoichiometric salt comprising eight molecules of irinotecan per one molecule of sucrosofate.

[00138] Embodiment 1: A storage stabilized liposomal irinotecan composition having a pH of 7.00-7.50 and comprising a dispersion of irinotecan liposomes encapsulating irinotecan sucrose octasulfate in vesicles consisting of cholesterol and the phospholipids 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE), at a concentration of irinotecan moiety equivalent to, in grams of irinotecan free anhydrous base, 500 mg (±10%) irinotecan moiety per mmol total liposome phospholipid and 4.3 mg irinotecan moiety per mL of the liposomal irinotecan composition, the storage stabilized liposomal irinotecan composition stabilized to form less than 20 mol% Lyso-PC during the first 6 months of storage at 4 °C.

[00139] Embodiment 2: A storage stabilized liposomal irinotecan composition having a pH of 7.00-7.50 and comprising a dispersion of irinotecan liposomes encapsulating irinotecan sucrose octasulfate in unilamellar bilayer vesicles consisting of cholesterol and the phospholipids 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE), at a concentration of irinotecan moiety equivalent to, in grams of irinotecan free anhydrous base, 500 mg (±10%) irinotecan moiety per mmol total liposome phospholipid and 4.3 mg irinotecan moiety per mL of the liposomal irinotecan composition, the storage stabilized

liposomal irinotecan composition having an irinotecan/sulfate compound gram-equivalent ratio of 0.85-1.2.

[00140] Embodiment 3: A storage stabilized liposomal irinotecan composition stabilized to form less than 20 mol% Lyso-PC during the first 6 months of storage at 4 °C, the liposomal irinotecan composition made by a process comprising steps of:

(a) forming a lipid dispersion in a solution made from TEA_8SOS and/or DEA_8SOS having a sulfate concentration of from 0.4 to 0.5 M and a pH between from 5 to 7, the lipids in said dispersion being DSPC, cholesterol and MPEG-2000-DSPE in an about 3:2:0.015, respectively, mole ratio;

(b) extruding the lipid dispersion between 60-70 °C through at least one 0.1 μm membrane to form liposomes;

(c) substantially removing ions derived from TEA_8SOS and/or DEA_8SOS that are outside the liposomes;

(d) contacting the liposomes at a temperature between 60-70 °C with a solution made using irinotecan free base or irinotecan salt, thereby forming a preparation of liposomes encapsulating irinotecan;

(e) substantially removing substances derived from the TEA_8SOS and/or DEA_8SOS and irinotcan ingredients that are outside the liposomes; and

(f) adjusting the pH of the composition to be from 7.0 to 7.5.

[00141] Embodiment 4: The liposomal irinotecan composition of any one of embodiments 1-3, made by a process comprising steps of:

(a) forming a lipid dispersion in a solution made from TEA_8SOS having a sulfate concentration of from 0.4 to 0.5 M and a pH between from 5 to 7, the lipids in said dispersion being DSPC, cholesterol and MPEG-2000-DSPE in an about 3:2:0.015, respectively, mole ratio;

(b) extruding the lipid dispersion between 60-70 °C through at least one 0.1 μm membrane to form liposomes;

(c) substantially removing ions derived from TEA_8SOS that are outside the liposomes;

(d) contacting the liposomes at a temperature between 60-70 °C with a solution made using irinotecan free base or irinotecan salt, thereby forming a preparation of liposomes encapsulating irinotecan;

(e) substantially removing substances derived from the TEA_8SOS and irinotcan ingredients that are outside the liposomes; and

(f) adjusting the pH of the composition to be from 7.0 to 7.5.

[00142] Embodiment 5: The liposomal irinotecan composition of embodiment 4, wherein the lipid dispersion is extruded through at least two stacked 0.1 μm polycarbonate membranes.

[00143] Embodiment 6: The liposomal irinotecan composition of any one of the previous embodiments, where the liposomes have a mean size of 110 nm as determined by dynamic light scattering and where the size is determined by the method of cumulants.

[00144] Embodiment 7: The liposomal irinotecan composition of any one of the previous embodiments, having a total irinotecan moiety content equivalent to of 4.3 mg/ml irinotecan free base anhydrous.

[00145] Embodiment 8: The liposomal irinotecan composition of any one of embodiments 3-6, wherein:

in step (a) the liposomes are formed from TEA_8SOS having a sulfate concentration of between 0.43-0.47 M; and

in step (d) the solution made using irinotecan free base or an irinotecan salt has an irinotecan moiety content equivalent to 500 g (±10%) of irinotecan free base anhydrous per mole of DSPC; and

in step (f) adjusting the pH of the composition to be from 7.2 to 7.3.

[00146] Embodiment 9: The liposomal composition of any one of the previous embodiments, containing less than 1 mol% lyso-phosphatidylcholine (lyso-PC) prior to storage at about 4 °C, and 20 mol% or less (with respect to total liposome phospholipid) of lyso-PC after 180 days of storage at about 4 °C.

[00147] Embodiment 10: The liposomal composition of embodiment 9, containing 20 mol% or less (with respect to total liposome phospholipid) of lyso-phosphatidylcholine (lyso-PC) after 6, 9 or 12 months of storage at about 4 °C.

[00148] Embodiment 11: The liposomal irinotecan composition of any one of the previous embodiments, comprising a total of 6.1 to 7.5 mg DSPC/ml, 2 to 2.4 mg cholesterol /ml, and 0.11 to 0.13 mg MPEG-2000-DSPE/ml, all in an aqueous isotonic buffer.

[00149] Embodiment 12: The liposomal irinotecan composition of any one of the previous embodiments, wherein the liposomal irinotecan comprises the irinotecan liposomes in an isotonic HEPES aqueous buffer at a concentration of between 2 and 20 mM.

[00150] Embodiment 13: The liposomal irinotecan composition of any one of the previous embodiments, further comprising sodium chloride at a concentration of from 130-160 mM.

[00151] Embodiment 14: The liposomal irinotecan composition of any one of the previous embodiments, wherein the irinotecan encapsulated in the liposomes is in a gelated or precipitated state as a sucrose octasulfate salt.

[00152] Embodiment 15: The liposomal irinotecan composition of any one of the previous embodiments, wherein the irinotecan liposomes have a diameter of 95-115 nm, as measured by quasi-elastic light scattering.

[00153] Embodiment 16: The liposomal irinotecan composition of any one of the previous embodiments, comprising a total of 6.81 mg DSPC/ml, 2.22 mg cholesterol /ml, and 0.12 mg MPEG-2000-DSPE/ml, 4.05 mg/mL HEPES aqueous buffer and 8.42 mg sodium chloride/mL.

[00154] Embodiment 17: The liposomal irinotecan composition of any one of the previous embodiments, having a pH of 7.25, wherein the irinotecan liposomes have a diameter of 110 nm as measured by quasi-elastic light scattering.

[00155] Embodiment 18: The liposomal irinotecan composition of any one of the previous embodiments, forming less than 1 mg/mL lyso-phosphatidylcholine (lyso-PC) after 6 months of storage at about 4 °C.

[00156] Embodiment 19: The liposomal irinotecan composition of any one of the previous embodiments, made by a process comprising steps of:

(a) forming a lipid dispersion in a solution of TEA_8SOS having a sulfate concentration of about 0.45 M and a pH of about 6.5, the lipids in said dispersion consisting of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE) in a mole ratio of 3:2:0.015, respectively;

(b) extruding the lipid dispersion between 60-70 °C through at least one 0.1 μm membrane to form liposomes;

(c) removing ions derived from TEA_8SOS that are outside the liposomes;

(d) contacting the liposomes at a temperature between 60-70 °C with a solution made using irinotecan hydrochloride trihydrate, to form a preparation of liposomes encapsulating about 500 g (±10%) irinotecan per mol total liposome phospholipid;

(e) removing substances derived from the TEA_8SOS and irinotcan ingredients that are outside the liposomes; and

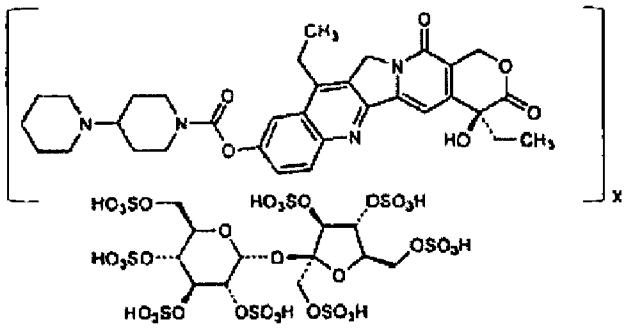
(f) adjusting the pH of the composition to be about 7.3.

[00157] Embodiment 20: The liposomal irinotecan composition of any of the previous embodiments, comprising a total of less than 100 ppm of TEA.

[00158] Embodiment 21: The liposomal irinotecan composition of any one of the previous embodiments, comprising a total of 30-100 ppm of TEA or DEA.

[00159] Embodiment 22: The liposomal irinotecan composition of any one of the previous embodiments, wherein at least 98% of the irinotecan is encapsulated in the irinotecan liposomes after 6 months of storage at about 4 °C.

[00160] Embodiment 23: The liposomal irinotecan composition of any one of the previous embodiments, comprising the irinotecan composition of formula (I) within the irinotecan liposomes, where x is 8:



(I).

EXAMPLES

[00161] The synthesis and characterization of several irinotecan liposome preparations is described in the following Examples. Unless otherwise indicated in the Examples, these irinotecan liposomes can be obtained by the following multi-step process. The invention therefore also provides methods of making irinotecan liposomes in line with the preparative methods set out in this subsection and in the Examples, and variations and combinations thereof.

[00162] First, liposome-forming lipids are dissolved in heated ethanol. These lipids included DSPC, cholesterol, and MPEG-2000-DSPE. Unless otherwise indicated, the DSPC, cholesterol, and MPEG-2000-DSPE are present in a 3:2:0.015 molar ratio. The resulting ethanol-lipid composition is dispersed in an aqueous medium containing substituted ammonium and polyanion under conditions effective to form a properly sized (e.g. 80-120 nm or 95-115 nm etc.), essentially unilamellar liposomes containing the substituted ammonium ion and polyanion trapping agent (SOS). The liposome dispersion can be formed, e.g., by mixing the ethanolic lipid solution with the aqueous solution containing a substituted ammonium ion and polyanion at the temperature above the lipid transition temperature, e.g.,

60-70 °C, and extruding the resulting lipid suspension (multilamellar liposomes) under pressure through one or more track-etched, e.g. polycarbonate, membrane filters with defined pore size, e.g. 50 nm, 80 nm, 100 nm, or 200 nm. Preferably the substituted ammonium is a protonated triethylamine (TEA) or diethylamine (DEA) and the polyanion is sucrose octasulfate (SOS), preferably combined in a stoichiometric ratio (e.g., TEA_8SOS). The concentration of the TEA_8SOS can be selected based on the amount of irinotecan loaded into the liposomes (e.g., to substantially or completely exhaust the concentration loading gradient across the liposome, and/or provide a liposome containing SOS and irinotecan in about a 1:8 mole ratio). For example, to prepare irinotecan SOS liposomes with 471g or 500 g irinotecan moiety/mol phospholipid, the TEA_8SOS used preferably has a concentration of about 0.4-0.5 M sulfate groups (e.g. 0.45 M or 0.475 M of sulfate groups, or 0.45 M or 0.475 M SOS). All or substantially all non-entrapped TEA or SOS is then removed (e.g., by gel-filtration, dialysis, or ultrafiltration/diafiltration).

[00163] The resulting trapping agent liposomes (e.g., encapsulating substituted ammonium compound such as TEA_8SOS or DEA_8SOS) are then contacted with an irinotecan solution under conditions effective to load the irinotecan into the trapping agent liposomes (i.e., conditions that allow the irinotecan to enter the liposome in exchange with TEA leaving the liposome). The irinotecan loading solution (e.g. at 15 mg/ml of anhydrous irinotecan-HCl, which can be prepared using corresponding amounts of irinotecan-HCl trihydrate) preferably contains an osmotic agent (e.g., 5% dextrose) and a pH of 6.5 (unless otherwise stated, pH values are mentioned in this specification were determined at room temperature). Drug loading is facilitated by increase of the temperature of the composition above the transition temperature of the liposome lipids (e.g., to 60-70 °C) to accelerate the transmembrane exchange of substituted ammonium compound (e.g., TEA) and irinotecan. In some embodiments, the irinotecan sucrosofate within the liposome is in a gelated or precipitated state.

[00164] The loading of irinotecan by exchange with substituted ammonium compound (e.g., TEA or DEA) across the liposome is preferably continued until all or substantially all of the substituted ammonium compound (e.g., TEA) is removed from the liposome, thereby exhausting all or substantially all of the concentration gradient across the liposome. Preferably, the irinotecan liposome loading process continues until the gram-equivalent ratio of irinotecan to SOS is at least 0.9, at least 0.95, 0.98, 0.99, or 1.0 (or ranges from about 0.9-1.0, 0.95-1.0, 0.98-1.0, or 0.99-1.0). Preferably, the irinotecan liposome loading process continues until at least 90%, at least 95%, at least 98%, or at least 99%, or more of the TEA is

removed from the liposome interior. In some embodiments of the present invention, the irinotecan SOS liposome composition prepared in this manner using TEA_8SOS contain less than 100 ppm TEA. In some embodiments of the present invention, the irinotecan SOS liposome composition prepared in this manner using TEA_8SOS contain 20-100 ppm, 20-80 ppm, 40-80 ppm, or 40-100 ppm TEA.

[00165] Extra-liposomal irinotecan and substituted ammonium compound (e.g., TEA or DEA) can be removed to obtain the final irinotecan liposome product. This removal can be facilitated by a variety of methods, non-limiting examples of which include gel (size exclusion) chromatography, dialysis, ion exchange, and ultrafiltration/diafiltration methods. The liposome external medium is replaced with injectable, pharmacologically acceptable fluid, e.g., buffered (pH between 7.1 to 7.5, preferably pH between 7.2 and 7.3) isotonic saline. Finally, the liposome composition is sterilized, e.g., by 0.2-micron filtration, dispensed into single dose vials, labeled and stored, e.g., upon refrigeration at 2-8 °C, until use. The liposome external medium can be replaced with pharmacologically acceptable fluid at the same time as the remaining extra-liposomal irinotecan and ammonium/substituted ammonium ion (e.g., TEA) is removed.

Quantification of Trapping Agent

[00166] For the purpose of the present invention, the liposomal trapping agent and substituted ammonium compound counter-ion (e.g., TEA_8SOS) is quantified based on the concentrations used for preparing the liposomes and calculated based on the number sulfate groups of the trapping agent. For example, a 0.1 M TEA_8SOS would be expressed herein as 0.8 M/L sulfate because each molecule of SOS has eight sulfate groups. In cases where a different trapping agent is used, this calculation would be adjusted, depending on the number of anionic groups (e.g., sulfate groups) per molecule of trapping agent.

Quantification of Lyso-PC in Irinotecan Liposome Preparations

[00167] The amount of lyso-PC in the irinotecan sucrose octasulfate liposome preparations tested to obtain data in FIGS. 11B and 12 was obtained by the HPLC method ("Method A"), which is described in Example 9.

[00168] A different preparative (TLC) method (herein, "Method B") was used obtain the lyso-PC measurements from Samples 1-23 herein, the lyso-phospholipid was determined by the following TLC method followed by phosphate analysis, rather than the HPLC method (Method A) discussed immediately above. The following steps were followed to measure

lyso-PC by Method B. An aliquot of liposome sample containing approximately 500 nmol phospholipid (PL) (e.g. 0.05mL of a 10 mM PL liposome solution) was desalted using a PD-10 column (GE Healthcare) equilibrated with water. The sample is eluted from the column with water and divided into three portions containing approximately 150 nmol of PL each, then dried under vacuum using a centrifugal concentrator (Savant Speed Vac Concentrator, Model#SVC100X). The dried lipids were dissolved in 30 µl of chloroform/methanol (5/1, vol/vol) and applied to the non-adsorbent region of a normal phase silica gel TLC plate (Uniplate by Analtech, cat # 44921) using a glass syringe. The TLC was run with a mobile phase consisting of chloroform/methanol/30% ammonium hydroxide/water (60/40/2.5/3.75, v/v/v/v) and the lipid visualized using iodine vapor. Determination of the PL was conducted by scraping the spots corresponding to phospholipid and lyso-phospholipid on the TLC into separate 12 × 75 mm borosilicate tubes for subsequent phosphate analysis.

[00169] The quantification of molar amounts of liposomally co-encapsulated irinotecan and sulfate compound is provided in the Examples.

Materials

[00170] For preparing samples 1-5 and 13 in Example 1 and samples 12 and 14-18 in Example 2, USP GMP grade irinotecan hydrochloride ((+)-7-ethyl-10-hydroxycamptothecine 10-[1,4'-bipiperidine]-1`-carboxylate, monohydrochloride, trihydrate, CAS Reg. No. 100286-90-6) was purchased from SinoPharm (Taipei, Taiwan); 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and methoxy-terminated polyethylene glycol (MW-2000)-distearoylphosphatidylethanolamine ((MPEG-2000-DSPE) were purchased from Avanti Polar Lipids (Alabaster, AL, USA); ultrapure cholesterol (Chol) was obtained from Calbiochem (La Jolla, CA, USA); and sucrose octasulfate was obtained from Euticals (Lodi, Italy).

[00171] For preparing samples 6-11 in Example 1, irinotecan hydrochloride trihydrate was obtained from PharmaEngine (Taiwan); 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and methoxy-terminated polyethylene glycol (MW-2000)-distearoylphosphatidylethanolamine ((MPEG-2000-DSPE) were purchased from Avanti Polar Lipids (Alabaster, AL, USA); ultrapure cholesterol (Chol) was obtained from Calbiochem (La Jolla, CA, USA); and sucrose octasulfate was obtained from Euticals (Lodi, Italy).

[00172] For preparing samples 19-23 in Example 8, Vinorelbine (VNB) was obtained from the pharmacy as a solution of vinorelbine tartate 10 mg/mL (Glaxo-SmithKline), and

topotecan (TPT) powder was obtained as a gift from Taiwan Liposome Company (Taipei, Taiwan).

[00173] All other chemicals, of analytical or better purity, were obtained from common suppliers.

[00174] Methods: The following methods were used in preparing Samples 1-5 and 13 (Example 1) and Samples 6-11 and 19-23 (Example 2), and Samples 12, and 14-18 (Example 3), to the extent not indicated otherwise below.

Triethylammonium Sucrose Octasulfate Preparation

[00175] Triethylammonium sucrose octasulfate (TEA_8SOS) and diethylammonium sucrose octasulfate (DEA_8SOS) were prepared from the sodium salt of sucrose octasulfate using ion exchange chromatography. Briefly, 15 g of sucrose octasulfate (sodium salt) was dissolved in water to give a sulfate concentration of 2.64 M. A Dowex 50W-8X-200 cation exchange resin was employed to prepare the acidic form of sucrose octasulfate. Defined resin was washed twice with 2 vol of 1 N NaOH, then with ddH_2O (doubly distilled water) to neutral pH, washed twice with 2 vol of 1 N HCl, and finally washed to neutral with ddH_2O and then repeated. A column was poured to a volume of 450 mL of resin and washed with 3 vol of 3 N HCl, and then rinsed with ddH_2O until the conductivity reaches less than 1 μS/cm. The sucrose octasulfate (sodium salt) solution (approximately 10 % of column capacity) was loaded on the column and eluted with ddH_2O. The column eluent was monitored using a conductivity detector to detect the elution of the sucrose octasulfate from the column. The acidic sucrose octasulfate was then titrated with triethylamine or diethylamine to a pH in between 6-7, and the sulfate content determined using a method modified from B. Sorbo et al., Methods in Enzymology, 143: 3-6, 1984 (see Sulfate Determination). The solution was finally diluted to a sulfate concentration corresponding to 0.65 M sulfate. The pH was typically in the range of 6-7. Residual sodium was determined using a sodium electrode, and any solution with residual sodium above 1 mol-% was not utilized further.

Sulfate Group Determination

[00176] Sulfate content in the sucrose octasulfate solutions was determined with a turbidimetric-based assay. Solutions consist of: (1) 15 g PEG 6000 and 1.02 g barium acetate in 100 mL water; (2) 142 mg sodium sulfate in 1 mL water; (3) Barium working solution: add dropwise 0.1 mL of the sodium sulfate solution to 100 mL barium solution while stirring. This solution should equilibrate for 1 hour before use and can be stored no longer than one

week; (4) 0.4 M trisodium citrate solution; (118 mg trisodium citrate/mL water); and (5) sulfate standard at 10 mM diluted in water from 1 N sulfuric acid. Using borosilicate test tubes the standards and solutions were made to a final volume of 100 µl. The standards were made in the range of 0.2-1 µmol sulfate (20-100 µl of the 10 mM standard). For samples of 0.6 M sulfate solution, a dilution of 1/100 and volume of 100 µl (0.6 µmol) was used. Each 100 µl sample/standard was treated with 100 µl of 70% perchloric acid and heated at 110-120 °C for 12 minutes. After cooling, 0.8 mL of the 0.4 M trisodium citrate solution was added followed by vortexing. A 0.25 mL volume from a stirring barium working solution was transferred to each tube and vortexed immediately. All samples/standards were allowed to equilibrate for 1 hour followed by vortexing and measurement of the absorbance at 600 nm. A linear standard curve of SO_4 concentrations versus OD600 was used to determine unknown SO_4 concentrations.

Sucrose Octasulfate Determination by HPLC

[00177] The concentration of sucrose octasulfate (mg/mL) in a sample can be calculated based on the area of the sucrose octasulfate peak produced from a standard of known concentration. The calculated concentration of the sucrose octasulfate is then used to calculate the concentration of sulfate (mM) in a sample.

[00178] The sample to be analyzed is chromatographed by HPLC using a Phenomenex, Bondclone 10 µ NH_2, 300 x 3.90 mm, PN 00H-3128-C0, or Waters µBondapak NH_2 10 µm 125 Å, (3.9 mm x 300 mm), Part No. WAT084040 using a mobile phase of 0.60 M ammonium sulfate, pH 3.0 eluted at 1.00 mL/min at a column temperature of 40 °C. Samples are detected by a refractive index detector, which is also at 4 0°C, for example, using an Agilent HPLC with Refractive Index Detector. USP Potassium Sucrose Octasulfate heptahydrate is used as a reference standard; CAS 76578-81-9, CAT No. 1551150.

[00179] The SOS assay standard and assay control samples are integrated using a baseline to baseline integration. The TEA-SOS samples are then integrated using a baseline to baseline integration. This may be performed manually beginning the baseline before the void volume valley to the end of the SOS tail, then dropping a line at the start of the TEA peak and the low point between the two peaks. Note: If a single baseline beginning before the void volume valley to the end of the SOS tail crosses the low-point between TEA and SOS peaks, two separate lines may be used that will approximate the baseline to baseline approach. TEA-SOS samples will show a TEA peak at a relative retention time of approximately 0.45 to the retention time of the SOS peak.

Drug Analysis

[00180] HPLC analysis of irinotecan was conducted on a Dionex system using a C_{18} reverse phase silica column (Supelco C_{18} column, 250 mm x 4 mm inner diameter, particle size of 5μm) preceded by a Supelco C_{18} guard column. A sample injection volume of 50 μl was used, and the column was eluted isocratically at a flow rate of 1.0 mL/min with a mobile phase consisting of 0.21 M aqueous triethylammonium acetate pH 5.5 and acetonitrile (73:27, v:v). Irinotecan and SN-38 typically eluted in 5.1 min and 7.7 min respectively. Irinotecan was detected by absorbance at 375 nm using a diode array detector, and SN-38 was detected by fluorescence (370 nm excitation and 535 nm emission).

Phosphate Determination

[00181] The following phosphate determination method was used for analyzing Samples 1-23. A modified Bartlett phosphate assay can be used to measure phospholipid (PL). Standards ranging from 10-40 nmol of phosphate were placed in 12 × 75 mm borosilicate tubes and treated exactly as the samples. Sulfuric acid (100 μl of 6 M H_2SO_4) was added to each tube placed in a heating block and heated to 180 °C for 45 minutes. Hydrogen peroxide (20 μl of a 30% solution) was added to each tube and then heated at 150 °C for 30 minutes. Ammonium molybdate (0.95 mL of a 2.2 g/l solution) and ascorbic acid (50 μl of a 10% aqueous solution) were subsequently added to each tube. After vortexing, the tubes were developed in boiling water for 15 minutes and then cooled to room temperature. For lysolipid analysis using thin-layer chromatography (TLC), the silica was pelleted by centrifugation at 1000 rpm for 5 minutes, and the blue color was measured in the supernatant by reading the absorbance at 823 nm. Samples not containing silica can eliminate the centrifugation step.

Drug Retention and Stability

[00182] Liposomal irinotecan stability (in terms of drug retention) was determined by separating the liposomal irinotecan from extraliposomal irinotecan using PD-10 (Sephadex G-25) size exclusion columns. Drug leakage was determined by comparison of the irinotecan (HPLC) to PL (described in Phospholipid Determination) ratio before and after separation of the extraliposomal irinotecan. Degradation of the irinotecan was determined by observation of additional peaks in the chromatogram after HPLC analysis. The irinotecan-to-phospholipid ratios and the drug encapsulation efficiencies are calculated using formulas 1 and 2 below, respectively.

(1) Irinotecan-to-phospholipid ratio (g Irinotecan/mol PL) = $\frac{\text{[Irinotecan] (mg/mL) *1000}}{\text{[phospholipid] (mM)}}$

(2) Encapsulation efficiency (%) = $\frac{\text{(Irinotecan-to-phospholipid ratio)AC}}{\text{(Irinotecan-to-phospholipid ratio)BC}}$

where (Irinotecan-to-phospholipid ratio)AC is the drug to phospholipid ratio after purification on the G-25 size exclusion column and (Irinotecan-to-phospholipid ratio)BC is the drug-to-phospholipid ratio before purification on the column.

Determination of encapsulated and free irinotecan in liposomal compositions

[00183] Liposomally encapsulated and free (non-encapsulated) irinotecan in the irinotecan sucrosofate liposomal compositions of Examples 3 and 4 was determined using a cartridge adsorption method. Oasis 60 mg 3 cc HLB cartridges (Waters) were conditioned by sequential passage of 2 mL methanol, 1 mL HEPES-buffered saline (HBS; 5 mM HEPES, 140 mM NaCl, pH 6.5), and 0.5 mL of 10% human serum albumin in normal saline, followed by 1 mL of HBS. Liposomal irinotecan sucrosofate compositions were diluted with normal saline to about 2.2 mg/mL irinotecan, and 0.5 mL aliquots were applied on the cartridges. The eluate was collected, the cartridges were rinsed with two portions of HBS (1.5 mL, 3 mL), and the rinses combined with the eluate to make a liposome fraction. The cartridges were additionally rinsed with 1.5 mL HBS and eluted with two 3-mL portions of methanol-HCl (90 vol.% methanol, 10 vol.% 18 mM HCl). The eluates were combined to make the free drug fraction. Liposomal drug fractions were transferred into 25-mL volumetric flasks, and free drug fractions were transferred into 10-mL volumetric flasks, brought to the mark with methanol-HCl, mixed well, and the liposome fraction flasks were heated for 10 minutes at 60 °C to solubilize the drug. Upon cooling, the solutions were filtered, and irinotecan was quantified in both fractions using reverse phase HPLC on a Phenomenex Luna C18(2) column, isocratically eluted with 20 mM potassium phosphate pH 3.0 methanol mixture (60:40 by volume) with UV detection at 254 nm. The drug peaks were integrated, and the amount of irinotecan in the samples was calculated by comparison to the linear standard curve obtained under the same conditions using irinotecan hydrochloride trihydrate USP reference standard. The drug encapsulation ratio was calculated as a percentage of encapsulated drug relative to the total of free and encapsulated drug in the sample.

pH Measurements

[00184] The pH was always measured at ambient temperature (i.e., 20-25 °C) using a potentiometric standard glass electrode method. The pH of liposome formulations was measured accordingly by putting the glass electrode into the liposome formulation and obtaining a pH reading.

Analysis of samples for TEA/DEA ppm

[00185] Samples analysis was performed by headspace gas chromatographic (GC) separation utilizing gradient temperature elution on a capillary GC column (50 m x 0.32 mm x 5 µm Restek Rtx-5 (5% phenyl-95% dimethylpolysiloxane)) followed by flame ionization detection (FID). A sample preparation and a standard preparation were analyzed, and the resulting peak area responses were compared. The amount of residual amine (e.g., TEA or diethyl amine (DEA)) was quantitated using external standards. In the case of TEA, the standard was ≥ 99%. Other reagents include Triethylene glycol (TEG), sodium hydroxide, and deionized (DI) water.

[00186] GC conditions were: carrier gas: helium; column flow: 20 cm/sec (1.24 mL/min); split ratio: 10:1 (which can be adjusted as long as all system suitability criteria are met); injection mode: split 10:1; liner: 2 mm straight slot (recommended but not required); injection port temperature: 140 °C, detector temperature: 260 °C (FID); initial column oven temperature: 40 °C; column oven temperature program:

rate (°C/min)	temperature (°C)	hold time (min)
n/a	40 0	0
2	100	0
20	240	17

54 min Runtime

[00187] Headspace Parameters: platen temperature: 90 °C; sample loop temperature: 100 °C; transfer line temperature: 100 °C; equilibration time: 60 minutes; injection time: 1 minute; vial pressure: 10 psi; pressurization time: 0.2 minute; shake: on (medium); injection volume: 1.0 mL of headspace; GC Cycle Time: 60 minutes (recommended but not required).

[00188] If no TEA is detected, report as "none detected;" if TEA results are < 30 ppm, report as < QL (30 ppm); of TEA results are ≥ 30 ppm, report to a whole number.

Determination of Liposome Size

[00189] Liposome particle size was measured using dynamic light scattering (DLS) using a Malvern ZetaSizer Nano ZS™ or similar instrument in aqueous buffer (e.g., 10 mM NaCl, pH 6.7) at 23-25°C using the method of cumulants. The z-average particle size and the polydistersity index (PDI) were recorded. The instrument performance was verified using Nanosphere NIST traceable standard of 100 nm polymer (Thermo Scientific 3000 Series Nanosphere Size Standard P/N 3100A, or equivalent with a certificate of analysis that includes Hydrodynamic Diameter). As used herein, "DLS" refers to dynamic light scattering and "BDP" refers to bulk drug product.

Example 1: *Effects of SOS Trapping Agent Concentration and pH onLiposomal Irinotecan Preparation Storage Stability*

[00190] The aim of this study was to determine, among other things, any changes in the physical and chemical stability of liposomes encapsulating irinotecan and sucrose octasulfate (SOS) trapping agent when stored at about 4 °C for certain periods of time. For this study, the liposomal concentration of the SOS trapping agent was reduced, while the ratio of 471 g irinotecan moiety per total mols of phospholipid was maintained.

[00191] A series of irinotecan SOS liposome preparations were prepared in a multistep process using different concentrations of SOS trapping agent and adjusting the pH of the final liposomal preparation to different pH values. Each of the irinotecan SOS liposome preparations contained irinotecan moiety concentration equivalent to 5 mg/mL irinotecan hydrochloride trihydrate. Irinotecan SOS liposome preparations of Samples 1-5 and 13 were prepared by a multi-step process of Example 1.

[00192] DSPC, cholesterol (Chol), and PEG-DSPE were weighed out in amounts that corresponded to a 3:2:0.015 molar ratio, respectively (e.g., 1264 mg/412.5 mg/22.44 mg). The lipids were dissolved in chloroform/methanol (4/1, v/v), mixed thoroughly, and divided into 4 aliquots (A-D). Each sample was evaporated to dryness using a rotary evaporator at 60 °C. Residual chloroform was removed from the lipids by placing under vacuum (180 µtorr) at room temperature for 12 hours. The dried lipids were dissolved in ethanol at 60 °C, and pre-warmed TEA_8SOS of appropriate concentration was added so that the final alcohol content was 10% (v/v). The lipid concentration was approximately 75 mM. The lipid dispersion was extruded at about 65 °C through 2 stacked 0.1 µm polycarbonate membranes (Nuclepore™) 10 times using Lipex thermobarrel extruder (Northern Lipids, Canada), to produce liposomes with a typical average diameter of 95-115 nm (determined by quasielastic light scattering; see

subsection "Determination of Liposome Size"). The pH of the extruded liposomes was adjusted as needed to correct for the changes in pH during the extrusion. The liposomes were purified by a combination of ion-exchange chromatography and size-exclusion chromatography. First, Dowex™ IRA 910 resin was treated with 1 N NaOH, followed by 3 washes with deionized water, and then followed by 3 washes of 3 N HCl, and then multiple washes with water. The liposomes were passed through the prepared resin, and the conductivity of the eluted fractions was measured by using a flow-cell conductivity meter (Pharmacia, Uppsala, Sweden). The fractions were deemed acceptable for further purification if the conductivity was less than 15 μS/cm. The liposome eluate was then applied to a Sephadex G-75 (Pharmacia) column equilibrated with deionized water, and the collected liposome fraction was measured for conductivity (typically less than 1 μS/cm). Cross-membrane isotonicity was achieved by addition of 40% dextrose solution to a final concentration of 5% (w/w) and the buffer (Hepes) added from a stock solution (0.5 M, pH 6.5) to a final concentration of 10 mM.

[00193] A stock solution of irinotecan was prepared by dissolving irinotecan•HCl trihydrate powder in deionized water to 15 mg/mL of anhydrous irinotecan-HCl, taking into account water content and levels of impurities obtained from the certificate of analysis of each batch. Drug loading was initiated by adding irinotecan in an amount of 500g irinotecan HCl anhydrous (corresponding to 471 g irinotecan free base anhydrous) per mol liposome phospholipid and heating to 60 ± 0.1 °C for 30 minutes in a hot water bath. The solutions were rapidly cooled upon removal from the water bath by immersing in ice cold water. Extraliposomal drug was removed by size exclusion chromatography, using Sephadex G75 columns equilibrated and eluted with Hepes buffered saline (10 mM Hepes, 145 mM NaCl, pH 6.5). The samples were analyzed for irinotecan by HPLC and phosphate by the method of Bartlett (see subsection "Phosphate Determination"). For storage, the samples were divided into 4 mL aliquots, and the pH was adjusted using 1 N HCl or 1 N NaOH, sterile filtered under aseptic conditions, and filled into sterile clear glass vials that were sealed under argon with a Teflon® lined threaded cap and placed in a thermostatically controlled refrigerator at 4 °C. At defined time points, an aliquot was removed from each sample and tested for appearance, liposome size, drug/lipid ratio, and drug and lipid chemical stability.

[00194] With respect to Example 1, liposome size distribution was determined in the diluted samples by dynamic light scattering using Coulter Nano-Sizer at 90 degree angle and presented as Mean ± Standard deviation (nm) obtained by the method of cumulants.

[00195] Irinotecan liposome preparations of samples 1-5 and 13 were further obtained as follows. The freshly extruded liposomes comprised two groups each incorporating TEA_8SOS as the trapping agent at the concentrations of (A) 0.45 M sulfate group (112.0±16 nm), (B) 0.475 M sulfate group (105.0±16 nm), (C) 0. 5 M sulfate group (97±30 nm), and (D) 0.6 M sulfate group (113±10nm). Samples 1-5 and 13 were loaded at an initial ratio of 471 g irinotecan free base anhydrous per mol total liposome phospholipids and purified as described above in the Example 1 description (equivalent to 500 g irinotecan HCl anhydrous). Samples 1, 5 and 13 were derived from extruded sample (A); sample 2 was from extruded sample (B); samples 3 and 4 were from extruded samples (C) and (D), respectively. Following purification, pH adjustment was made using 1 N HCl or 1 N NaOH prior to sterilization and the filling of the vials. Data from samples 1-5 are shown in Table 7 (Example 1), and data from sample 13 is shown in Table 8 (Example 2).

[00196] Irinotecan liposome preparations of samples 6-11 were further obtained as follows. The freshly extruded liposomes comprised two groups each incorporating TEA_8SOS as the trapping agent at the concentrations of (A) 0.45 M sulfate group (116±10 nm) and (B) 0.6 M sulfate group (115.0±9.0 nm). Samples 6-8 were derived from extruded sample (A), and samples 9-11 were from extruded sample (B). Following purification, pH adjustment was made if necessary by addition of 1 N HCl or 1 N NaOH as appropriate. Sample 12 was prepared as described in Example 2 and is included in Table 7 for comparative purposes.

[00197] Irinotecan liposomes with the extra-liposomal pH values, irinotecan free base concentration (mg/mL) and various concentrations of sucrose octasulfate for certain irinotacne liposome compositions are listed in Table 6 (6 months storage at 4 degrees C) and Table 7 below, and were prepared as provided in more detail as described herein.

[00198] FIGS. 4A-4C are plots showing the mol% of lyso-PC in irinotecan liposome preparations selected from Table 7 having a pH of greater than 6.5 (i.e., 7.25 or 7.5 as indicated in each FIG.). Lyso-PC was determined with Method B (TLC) disclosed herein, after storage of each sample at 4 °C for the first 1, 3, 6, and/or 9 months. These plots include a linear regression line to the data for each Sample, as an estimate for the rate of increase in lyso-PC (mol%) over time in each sample. Surprisingly, increasing the pH of the irinotecan liposome preparations above 6.5 (e.g., 7.25 and 7.5) decreased the amount of Lyso-PC measured during refrigerated storage at 4 °C compared to irinotecan liposomes formed at comparable Stability Ratios. This trend was apparent at various concentrations of liposomal irinotecan. For example, with respect to liposomal irinotecan compositions prepared at a strength of about 4.3 mg irinotecan moiety/mL, the mol% lyso-PC levels measured in

Samples 5 and 7 were significantly lower at all data points (after the first 1, 6 and 9 months of storage at 4 °C after manufacturing) compared to the mol% lyso PC levels measured for Sample 1 at pH 6.5 (data in Table 7). Similarly, with respect to liposomal irinotecan compositions prepared at a strength of about 18.8 mg irinotecan moiety/mL, the mol% lyso-PC levels measured in Sample 13 was significantly lower at all data points (after the first 1 and 9 months of storage at 4 °C after manufacturing) compared to the mol% lyso PC levels measured for either Sample 12 or Sample 14 at pH 6.5 (data in Table 8).

Table 6: *Lyso-PC Measurements after 6 Months of Refrigerated Storage*

Sample	pH	Drug (mg/mL)	Irinotecan (g) / mol PL	[sucrosofate] mM	%lyso-PC (180 d)	Lyso PC Stability Ratio
1	6.5	4.7	471	56.25	19.5	1047
2	6.5	4.7	471	59.375	17	992
4	6.5	4.7	471	75	30.2	785
5	7.25	4.7	471	56.25	7.1	1047
6	6.5	4.7	471	56.25	14.6	1047
7	7.25	4.7	471	56.25	7.4	1047
8	7.5	4.7	471	56.25	5.4	1047
9	6.5	4.7	471	75	29.8	785
10	7.25	4.7	471	75	24.1	785
11	7.5	4.7	471	75	22.8	785
13	7.25	4.7	471	56.25	9.7	1047

[00199] Additional results from comparative stability studies in Example 1 are provided in Table 7 below. The mol% of lyso-PC was determined after storing the liposome preparations at 4 °C for 1, 3, 6, 9, and/or 12 months, as indicated in Table 7. For each sample, Table 7 provides the concentration of SOS used to prepare the liposome, expressed as molar concentration of sulfate groups (one molecule of SOS includes 8 sulfate groups). Unless otherwise indicated, all of the irinotecan liposomes in Table 7 were prepared using an irinotecan moiety (as explained above, based on the free base anhydrous) to total phospholipid ratio of 471 g irinotecan moiety (equivalent to the amount of irinotecan moiety in 500 g anhydrous irinotecan HCl salt) per mole total liposome phospholipid, respectively. Table 7 also contains the stability ratio for each sample, calculated as the ratio of 471 g irinotecan moiety (based on the free base anhydrous) per mol phospholipid, divided by the concentration of sulfate groups in moles/L used to prepare the liposomes. The liposomes of the samples described in Table 7 each had a measured size (volume weighted mean) of

between about 89-112 nm and an irinotecan encapsulation efficiency of at least 87.6%. Encapsulation efficiency was determined in accordance with subsection "Drug Retention and Stability."

Table 7: *Irinotecan Liposome Preparations with Various Stability Ratios and pH (liposome vesicles formed from DSPC, cholesterol (Chol), and PEG-DSPE in a 3:2:0.015 molar ratio)*[c]

Sample	pH	Molar concentration of sulfate groups in the sucrosofate entrapped in the liposomes	Stability Ratio	Time (months)	Mol % Lyso-PC	Size	%SN38
12	6.5	0.65 M	724	0	3.8 (±0.6)	110.3±19.7	
				1	18.3 (±1.2)	120.1±12.3	0.5
				3	32.7 (±1.9)	107.6±20.2	0.3
				9	35.4 (±0.5)	101.2±26.0	0.3
				12	37.9	106.4±26.0	0.2
4	6.5	0.60 M	785	1	10.1 (±0.3)	107.6±12.4	0.030
				3		109.5±13.0	0.014
				6	30.2 (±0.9)	105.3±17.7	0
				9	35.8 (±0.6)	105.7±27.9	0.005
9	6.5	0.60 M	785	1	11.3 (±0.8)	107.6±26.6	
				3	22.1 (±1.3)	108.6±13.4	0.016
				6	29.8 (±1.9)	112.6±9.4	0.010
				9	34.7 (±1.2)	111.1±15.2	0.005
10	7.25	0.6 M	785	1	9.6 (±0.8)	98.9±7.0	
				3	16.9 (±1.1)	108.4±11.8	0.011
				6	24.1 (±0.8)	103.0±8.9	0.010
				9	29.0 (±0.6)	105.9±23.8	0.005
11	7.5	0.60 M	785	1	9.33 (±0.5)	102.2±23.6	
				3	17.1 (±5.01)	102.6±9.8	0.012
				6	22.8 (±0.7)	105.9±18.1	0.010
				9	28.7 (±3.1)	112.4±15.3	0.005
3	6.5	0.50 M	942	1	9.9 (±0.2)	109.7±13.7	0.024
				3		104.7±12.6	0.014
				6	26.5 (±0.3)	106.6±12.7	0
				9	35.7 (±0.6)	88.5±36.5	0.006
2	6.5	0.475 M	992	1	5.7 (±0.2)	89.4±31.9	0.028
				3		84.9±33.8	0.018
				6	17.0 (±0.4)	93.4±26.0	
				9	23.6 (±1.0)	102.6±18.8	0.006
1	6.5	0.45 M	1047	1	5.0 (±0.1)	108.5±13.6	0.036
				3		98.6±31.3	0.022
				6	19.5 (±0.6)	112.6±11.4	0
				9	25.4 (±0.6)	93.8±27.90	0
6	6.5	0.45 M	1047	1	5.6 (±1.37)	106.7±18.2	
				3	9.6 (±1.4)	96.4±26.0	0.051
				6	14.6 (±0.5)	98.2±24.0	0.01
				9	17.4 (±0.4)	109.2±12.6	0.006
5	7.25	0.45 M	1047	1	2.0 (±0.3)	106.4±18.5	0.033
				3		103.9±18.8	0.015

Sample	pH	Molar concentration of sulfate groups in the sucrosofate entrapped in the liposomes	Stability Ratio	Time (months)	Mol % Lyso-PC	Size	%SN38
				6	7.1 (±0.4)	107.2±17.3	0
				9	11.1 (±0.1)	100.0±28.1	0.007
7	7.25	0.45 M	1047	1	3.2(±0.3)	105.3±13.1	
				3	3.8 (±0.5)	104.1±16.7	0.022
				6	7.4 (±0.5)	105.5±13.4	0.010
				9	8.1 (±0.7)	107.3±13.0	0.006
8	7.5	0.45 M	1047	1	2.2 (±0.1)	102.8±14.2	
				3	2.8 (±0.1)	103.5±11.1	0.018
				6	5.4 (±0.2)	91.8±28.6	0.010
				9	7.1 (±1.2)	108.2±19.0	0.006

[c] Measured according to Method B, as described herein.

[00200] The results from this storage stability study demonstrated that a reduction of the concentration of SOS trapping agent (measured as the molar concentration of sulfate) used in the preparation of the liposomes, while the ratio of irinotecan free base anhydrous (in g) to total liposome phospholipid (in mol) was kept constant, resulted in greater storage stability of the irinotecan SOS liposomes, as measured by the amount of lyso-PC detected in the irinotecan liposome preparation after 6 and 9 months of refrigerated storage at 4 °C. In liposome preparations manufactured to a pH of 6.5 (see "pH Measurements" method described herein), reducing the concentration of SOS trapping agent during liposome manufacture lead to a reduction of amounts of lyso-PC detected in liposome preparations after storage at 4 °C.

[00201] Without being bound by theory, it is believed that once purified from the extraliposomal trapping agent during preparation, the interior space of the liposome is acidified. This may be due to the redistribution of the amine component of the trapping agent salt from inside to the outside of the liposome following removal of extraliposomal TEA_8SOS, with a deposition of a hydrogen ion intraliposomally at each occurrence. Added drug, such as irinotecan, capable of protonation, also distributes between the exterior and the interior space of the liposome. Protonation of the drug distributed in the interior of the liposome and binding of the protonated drug to sucrosofate effects intraliposomal loading of drug and results in a reduction in the intraliposomal concentration of both TEA and hydrogen ions, decreasing the extent of intraliposomal acidification.. In the case of irinotecan liposome it is postulated that at a drug load of 500 g irinotecan hydrochloride (ie. 471 mg irinotecan) /mol liposome phospholipid with SOS at a sulfate concentration of 0.6 M, there is incomplete

exhaustion of the excess intraliposomal TEA. While not being the basis for retaining the drug in the liposome, this may provide for an acidic liposomal interior, which may contribute to the degradation of the drug and lipid components of the liposome as seen with samples 7 and 13. In contrast, samples 8 and 5 have identical drug loads of 500 g irinotecan hydrochloride (ie. 471 mg irinotecan moiety) /mol, but lower SOS concentrations of 0.45 M sulfate and 0.475 M sulfate, respectively. In these particular cases, the levels of lysolipid measured are lower. Finally, it is apparent that the most stable liposome formulation combines the higher drug/trapping agent ratios with the higher external pH (i.e., pH 7.25).

[00202] The irinotecan liposomes of samples 1-11 retained good colloidal stability up to 9 months at 4 °C, as judged by the absence of precipitation and the relatively narrow and reproducible particle size distributions, where the irinotecan moiety concentration corresponding to 4.71 mg/mL irinotecan free base anhydrous. Irinotecan was efficiently and stably entrapped with minimal leakage (<10%) over extended periods of storage (see "Drug Retention and Stability" method described herein).

[00203] Samples 1 and 2 had identical initial loads of about 471 g irinotecan moiety (as explained above, based on the free base anhydrous) per mole phospholipid, but lower SOS concentrations of 0.45 M sulfate groups and 0.475 M sulfate groups, respectively. Similarly, samples 6, 7, and 8 had a lower SOS concentration of 0.45 M sulfate but the same drug load of 471 g irinotecan moiety (as explained above, based on the free base anhydrous)/mol phospholipid, result in a considerably lower lyso-lipid content (7-17% after 9 months).

[00204] Increased levels of lyso-PC were measured in samples at pH of 6.5 regardless of the drug load or trapping agent concentrations during liposome manufacture, reaching up to 35 mol% of the phospholipid for some samples (1, 2, and 3). Adjustment of pH to 7.25 rendered the liposomes less susceptible to lyso-PC formation, with levels reaching 9.72 % of total PC (e.g., compare lyso-PC levels in samples 1 and 13). Samples with higher drug to trapping agent concentration ratios and higher pH formed less lyso-lipid, as seen in samples 7 and 8 having 7-8 mol% lyso-lipid after 9 months. The combination of a higher drug trapping agent ratio and higher pH (e.g., compared to Sample 12) reduced lyso-lipid formation. The most stable liposome formulation combines the higher drug/trapping agent ratios (i.e. Stability Ratios above 942 defined with respect to the amount of irinotecan free base) with the higher external pH above 6.5 (e.g., comparing samples 1 and 13).

[00205] Furthermore, the % SN38 measured in the irinotecan liposome preparations 1-11 over 9 months was not greater than about 0.05% SN38 (i.e., relative amount of SN38 by comparison to irinotecan and SN38), while sample 12 irinotecan liposome preparation had

from 0.20-0.50% SN38 measured over the same time period (determined by "Drug Analysis" method described herein). In each of samples 1-5 and 13, irinotecan was stably entrapped with low leakage from liposomes (less than 13%; determined by "Drug Retention and Stability" method described herein) and low conversion to the active cytotoxic SN-38, less than 0.1%, and in samples stored at higher pH (7.25), less than 0.05%.

Example 2: *Increasing Concentration of Irinotecan Liposomes in a Liquid Preparation*

[00206] The aim of this storage stability study was to determine any changes in the physical and chemical stability of liposomal irinotecan SOS when stored at 4 °C. During this study, the concentration of the sucrose octasulfate (SOS) trapping agent used for liposome preparation was kept at a sulfate group concentration of 0.65 M, while varying: (1) the initial counter ion of the SOS trapping agent during the preparation of the irinotecan liposomes (using TEA_8SOS or DEA_8SOS), (2) the ratio of the amount of irinotecan free base anhydrous (in gram) to phospholipid (in mol) (about 471 g or 707 g irinotecan moiety (as explained above, based on the free base anhydrous) per mole phospholipid), (3) the concentration of the irinotecan free base anhydrous in the liquid irinotecan preparation (4.7 mg/mL or 18.8 mg/mL encapsulated irinotecan (based on the equivalent concentration of irinotecan moiety from irinotecan hydrochloride trihydrate) in the liquid irinotecan liposome preparation), (4) the pH to which the irinotecan liposome preparation was adjusted (pH 6.5, or 7.25), and (5) the buffer of the irinotecan liposome preparation (HEPES or histidine).

[00207] The formulation parameters investigated include: liposome size, drug to phospholipid ratio in the irinotecan liposomes, the irinotecan drug encapsulation efficiency and general appearance, the presence of irinotecan degradation products, and lyso-PC (in mol%) formation.

[00208] A series of irinotecan SOS liposome preparations were prepared in a multistep process using different concentrations of SOS trapping agent relative to encapsulated irinotecan and adjusting the pH of the final liposomal preparation to different pH values. DSPC, cholesterol (Chol), and PEG-DSPE were weighed out in amounts that corresponded to a 3:2:0.015 molar ratio, respectively (730.9 mg/238.5 mg/13.0 mg). The lipids were dissolved in chloroform/methanol (4/1, v/v), mixed thoroughly, and divided into 2 aliquots. Each sample was evaporated to dryness using a rotary evaporator at 60 °C. Residual chloroform was removed from the lipids by placing under vacuum (180 μtorr) at room temperature for 12 hours. The dried lipids were dissolved in ethanol at 60 °C, and pre-warmed TEA_8SOS or DEA_8SOS (at a concentration of 0.65 M sulfate group) was added so that the final alcohol

content was 10% (v/v) and the samples were designated A and B, respectively. The lipid concentration was approximately 75 mM. The lipid dispersion was extruded through 0.1 μm polycarbonate membranes (Nuclepore™) 10 times, to produce liposomes with a typical average diameter of 95-115 nm. The pH of the extruded liposomes was adjusted as needed (with 1 N NaOH) to the selected preparation pH. The liposomes were purified by a combination of ion-exchange chromatography and size-exclusion chromatography. First, Dowex™ IRA 910 resin was treated with 1 N NaOH, followed by 3 washes with deionized water, and then followed by 3 washes of 3 N HCl, and then multiple washes with water. The conductivity of the eluted fractions was measured by using a flow-cell conductivity meter (Pharmacia, Uppsala, Sweden). The fractions were deemed acceptable for further purification if the conductivity was less than 15 μS/cm. The liposome eluate was then applied to a Sephadex G-75 (Pharmacia) column equilibrated with deionized water, and the collected liposome fraction was measured for conductivity (typically less than 1 μS/cm). Cross-membrane isotonicity was achieved by addition of 40% dextrose solution to a final concentration of 5% (w/w), and the buffer (Hepes) was added from a stock solution (0.5 M, pH 6.5) to a final concentration of 10 mM.

[00209] A stock solution of irinotecan was prepared by dissolving 326.8 mg irinotecan•HCl trihydrate powder in 20.0 mL deionized water to 15 mg/mL of anhydrous irinotecan-HCl, taking into account water content and levels of impurities obtained from the certificate of analysis of each batch. Drug loading was initiated by adding irinotecan free base anhydrous at 500g/mol or 750g/mol phospholipid and heating to 60 ± 0.1 °C for 30 min in a hot water bath. The solutions were rapidly cooled upon removal from the water bath by immersing in ice cold water. Extraliposomal drug was removed by size exclusion chromatography, using Sephadex G75 columns equilibrated and eluted with Hepes buffered saline (10 mM) (HBS), pH 6.5 for sample A and histidine buffered saline at pH 7.25 for sample B. The samples were analyzed for irinotecan by HPLC and phosphate by the method of Bartlett (see Phosphate Determination).

[00210] For storage, the samples were divided into 4 mL aliquots, and the pH was adjusted if necessary using 1 N HCl or 1 N NaOH, sterile filtered under aseptic conditions, and filled into sterile clear glass vials that were sealed under argon with a Teflon® lined threaded cap, and placed in a thermostatically controlled refrigerator at 4 °C. At defined time points, an aliquot was removed from each sample and tested for appearance, size, drug/lipid ratio, and drug and lipid chemical stability.

[00211] The liposome size was determined in the diluted samples by dynamic light scattering using Coulter Nano-Sizer at 90 degree angle and presented as Mean ± Standard deviation (nm) obtained by the method of cumulants.

[00212] The results from comparative stability studies are provided in Table 8 (for samples prepared using TEA_8SOS trapping agent starting material) and Table 9 (for samples prepared using DEA_8SOS trapping agent starting material).

Table 8: *Irinotecan Liposomes prepared with TEA_8SOS Trapping Agent in Hepes Buffer (10 mM)*[d]

Sample	Final Prep pH	[irinotecan]/ total mol PL	Molar concentration of sulfate groups in the sucrosofate entrapped in the liposomes	Stability ratio	[irinotecan] g/mol	Time (months)	Mol % Lyso-PC
12	6.5	471	0.65 M	724	5	0 1 3 9 12	3.8 (±0.6) 18.3 (±1.2) 32.7 (±1.9) 35.4 (±0.5) 37.9 (±0.5)
14	6.5	471	0.65 M	724	20	0 1 3 9 12	3.8 (±0.6) 15.9(±0.6) 19.2(±0.3) 32.1(±0.5) 36.0(±0.8)
13	7.25	471	0.45 M	1047	20	1 6 9	2.6 (±0.6) 9.72 (±1.9) 13.8 (±1.0)

[d] Measured according to Method B, as described herein.

[00213] Sample 13 (Example 2, Table 8) was stored at a concentration 4 fold greater (20 mg irinotecan/mL) than samples 1-5 (Example 1) and still retained good colloidal stability, with no observable aggregation or precipitation.

Table 9: *Irinotecan Liposomes prepared with DEA8SOS Trapping Agent at a sulfate group concentration of 0.65M, pH 7.25,*[e]

Sample	mg irinotecan /mL	[irinotecan]/ total mol PL	Stability Ratio	Time (months)	Mol % Lyso-PC	Size	% SN38
15	18.8	471	724	0	2.6 (±0.2)	106.8±18.3	
				1	8.8(±1.2)	106.3±26	0.05
				3	6.9(±0.8)	85.9±30.8	0.08
				9	9.6(±0.5)	97.1±19.0	0.05
				12	11.0(±0.4)	116.1±26.6	0.04
16	18.8	707	1086	0	2.0 (±0.6)	101.0±23.0	
				1	0.9(±0.1)	112.3±23.5	0.01
				3	0.93(±0.5)	93.2±25.0	0.09
				9	2.3(±0.1)	99.2±19.7	0.03
17	4.7	707	1086	0	2.0 (±0.6)	101.0±23	
				1	0.4 (±0.2)	112.6±23.3	0.07
				3	1.1 (±0.4)	102.4±16.2	0.05
				9	1.5 (±0.2)	99.5±15.8	0.06
				12	1.5 (±0.1)	106.2±22.5	0.04
18	18.8	707	1086	0	2.0 (±0.6)	101.0±23	
				1	0.7 (±0.3)	108.1±23.7	0.01
				3	0.4 (±0.4)	100.2±18.0	0.04
				9	0.1 (±0.1)	98.1±18.3	0.03
				12	1.5 (±0.1)	100.0±26.5	0.01

[e] Measured according to Method B, as described herein.

[00214] The freshly extruded liposome sizes encapsulated either (A) TEA8SOS at 0.65 M sulfate (113.0±23.8nm) or (B) DEA8SOS at 0.65 M sulfate groups (103.2±21.1nm) (the only exception being sample 13, which had 0.45 M sulfate groups). From (A), samples 12 and 14 and from sample (B) samples 15-18 were derived, with samples 12, 14, 15, and 16 being loaded at 471 g irinotecan free base anhydrous (equivalent to 500 g irinotecan HCl anhydrous) per mol total liposome phospholipids and samples 16-18 being loaded at 750 g irinotecan moiety (as explained above, based on the free base anhydrous) per mol phospholipid. Following purification, pH adjustment was made using 1 N HCl or 1 N NaOH as appropriate and as described in Tables 7 and 8 to either pH 6.5 or 7.25. Sample 12 was prepared as described in Example 1 and is included in Table 8 for comparison purposes.

[00215] The data showed that the liposomes retain good colloidal stability up to a year at 4 °C, as judged by the absence of precipitation and the relatively narrow and reproducible particle size distributions. Secondly, it is apparent that the colloidal stability was also good for more concentrated samples when stored at high pH and at elevated drug to phospholipid ratio, indicating that at irinotecan moiety concentrations equivalent to 20 mg/mL and 40 mg/mL of irinotecan hydrochloride trihydrate, the liposomes are stable and resist formation of aggregates.

[00216] In all cases, irinotecan was stably entrapped in liposomes with low leakage and low conversion to the active cytotoxic SN-38 (i.e., relative amount of SN38 by comparison to irinotecan and SN38); less than 0.5 mol% in all cases, and with the exception of sample 12, less than 0.1 mol% SN-38. Data were obtained by "Drug Retention and Stability" method and "Drug Analysis" method described herein.

[00217] Increased levels of lyso-PC were measured in samples that had been adjusted to pH 6.5 and prepared at a ratio of 471 g irinotecan moiety (as explained above, using an equivalent amount of 500 g irinotecan HCl anhydrous) per mole of phospholipid, reaching 36-37 mol% (of the total phosphatidylcholine) for samples 12 and 14, whereas adjustment of the pH to 7.25 rendered the liposomes less susceptible to lyso-lipid formation, with lyso-PC levels approaching only 11 mol% (of the total phosphatidylcholine) after one year for Sample 15.

[00218] Changing the liposomal pH from 6.5 to 7.25 had no detrimental effect on colloidal stability or drug leakage.

Example 3: *Storage stability of stabilized irinotecan liposomes* with varying amounts of TEA (SOS trapping agent counter-ion)

[00219] Irinotecan liposomes were prepared by loading irinotecan into liposomes encapsulating sucrose octasulfate (SOS) and a substituted ammonium counter ion (e.g., protonated TEA). The effect of changing the residual amount of the substituted ammonium in the drug loaded irinotecan SOS liposome was evaluated by making multiple irinotecan SOS liposomes containing varying amounts of the encapsulated residual substituted ammonium ion, storing these irinotecan SOS liposomes under refrigeration at 4 °C for 6 months and then measuring the amount of Lyso-PC (in mol%) in these irinotecan SOS liposomes.

[00220] The data demonstrated that reducing the amount of substituted ammonium ion within irinotecan SOS liposomes results in lower levels of lyso-PC after 6 months of refrigerated storage at 4 °C. In particular, irinotecan SOS liposomes having less than 100 ppm (e.g., 20-100 ppm TEA) substituted ammonium exhibited lower levels of lyso-PC formation after 6 months of refrigerated storage 4 °C.

[00221] Six lots (Samples 24-29) of liposomal irinotecan sucrosofate were prepared according to certain embodiments of the invention, following the protocols described herein, having the Stability Ratios of 1046-1064, lipid composition of DSPC, cholesterol, and MPEG-2000-DSPE at the molar ratio of 3:2:0.015, respectively.

[00222] The amount of lyso-PC in Table 10 was determined by HPLC (Method A herein).

Table 10: *Irinotecan liposome preparations at pH 7.3 (irinotecan SOS encapsulated in vesicles formed from DSPC, cholesterol (Chol), and PEG-DSPE in a 3:2:0.015 molar ratio)*

Sample (lot)	D (mm)	Irinotecan mg/mL	DL ratio g/mol	pH	Irinotecan/ SOS gram equiv ratio	TEA ppm	Lyso PC initial mg/mL[f]	Lyso-PC rate mg/mL /month	mol% Lyso-PC at 180 days[g]
24 (1)	110	4.51	502	7.3	1.020±0.012	16	0.060	0.0077	2.2
25 (2)	109	4.38	517	7.3	1.018±0.031	14	0.059	0.0124	3.0
26 (3)	109	4.43	481	7.3	0.963±0.008	39	0.148	0.0309	6.9
27 (4)	107	4.43	469	7.3	0.965±0.019	79	0.081	0.0313	5.4
28 (5)	108	4.43	487	7.3	0.983±0.021	18	0.060	0.0126	2.8
29 (6)	112	4.43	503	7.3	0.907±0.009	100	0.110	0.0585	10.1

[f] Measured according to Method A, as described herein.

[g] Measured according to Method A, as described herein.

[00223] The liposomes (100-115 nm) were obtained by extrusion of the lipid dispersed in a TEA-SOS solution (0.4-0.5 M sulfate) through 100-nm polycarbonate membranes (Nuclepore), purified from extraliposomal TEA-SOS by tangential flow diafiltration buffer exchange against osmotically balanced dextrose solution, loaded with irinotecan by raising the temperature to 68 °C, and stirring for 30 minutes, quickly chilled, and purified from extraliposomal TEA and any unencapsulated drug by tangential flow diafiltration buffer exchange against buffered physiological sodium chloride solution. The irinotecan sucrosofate liposome composition was filter-sterilized by passage through the 0.2-μm membrane filters, aseptically dispensed into sterile glass vials, and incubated under refrigeration conditions (5 ± 3 °C). At the refrigerated storage times of approximately 0, 3, 6, 9, and in some cases, 12 months, duplicate vials of each lot were withdrawn and analyzed for the amount of accumulated lyso-PC using HPLC method with evaporative scattering detector. The liposome compositions were also characterized by the particle size, irinotecan and liposome phospholipid concentration, pH of the liposome composition, irinotecan/sucrosofate gram-equivalent ratio (Iri/SOS ratio) and residual triethylammonium (protonated TEA) (as triethylamine). The mean particle size (D) and polydispersity index (PDI) were determined by DLS method using Malvern ZetaSizer NanoZS™. Irinotecan concentration in the liposome compositions was determined by HPLC. Total phospholipid was determined spectrophotometrically by the blue phosphomolybdate method after digestion of the liposomes in sulfuric acid/hydrogen peroxide mixture.

[00224] Drug/lipid (DL) ratio was calculated by dividing the drug amount (as free base anhydrous) in g by the molar amount of liposome phospholipid in the liposome preparation.

Liposomally-entrapped SOS was quantified after passage of the liposomes through a Sephadex G-25 gel-chromatography column (PD-10, GE Healthcare) eluted with normal saline. To determine the Irinotecan/SOS gram-equivalent ratio, 0.1-mL aliquots of the eluted liposome fractions, in triplicate, were mixed with 0.05 mL of 70% perchloric acid,, hydrolyzed at 95-100 °C for 1 hour, neutralized with 0.8 mL of 1 M sodium acetate, filtered to remove insoluble lipid products, and the amount of sucrosofate-derived sulfate groups in the filtrates was quantified by turbidimetry using barium-PEG reagent essentially as described under Methods. Another set of triplicate aliquots of the same liposome eluates was lysed in 70% acidified (0.1M HCl) aqueous isopropanol and assayed for irinotecan by spectrophotometry at 365 nm. The irinotecan/sucrosofate gram-equivalent ratio (Iri/SOS ratio) was calculated in each eluted liposome fraction by dividing the measured molar concentration of the drug by the measured molar concentration of the sulfate groups. The pH was measured as described in subsection "pH Measurements." TEA was quantified by headspace gas chromatographic (GC) separation utilizing gradient temperature elution on a capillary GC column followed by flame ionization detection (FID). Results are expressed as ppm (parts per million) of TEA. Levels of TEA are determined by external quantitation against a standard.

[00225] The data in 5, 6, 7, 10, 11A, 11B, and 12 was obtained from liposomal irinotecan samples prepared by loading 0.4-0.5 M TEA_8SOS trapping agent liposomes with about 400-600 mg (e.g., about 500 g) irinotecan moiety per mol total phospholipid (Stability Ratios ranging from about 1000-1200) and a pH after manufacturing of about 7-.0-7.5 (e.g., about 7.25). The amount of lyso-PC in each of these liposomal irinotecan samples was measured at the time points indicated in FIG. 5-7 using the HPLC method of Example 9.

[00226] The lyso-PC accumulation data (in mg lyso-PC/mL liposome composition) were plotted against the storage time, as shown on FIG. 5 (Samples 24-26/Lots 1-3) or FIG. 6 (Samples 27-29/Lots 4-6). A linear correlation was observed, where the lyso-PC accumulation varied from about 0.008 mg/mL/month to about 0.06 mg/mL/month, the higher rates being characteristic for the compositions with higher TEA amounts. The amounts of lyso-PC accumulated at day 180 (about 6 months) of storage were determined from the linear approximation of the multi-point data (FIGS. 5A and 5B) and expressed as mol% of PC taking the molecular weight of lyso-PC equal to 523.7 g/mol. All six lots (Samples 24-29; see Table 10) accumulated less than 20 mol% of lyso-PC at day 180 of refrigerated storage. The lots with less than 20 ppm TEA and Iri/SOS gram equiv. ratio of more than 0.98 showed the least lyso-PC accumulation (less that about 0.015 mg/mL/month, lyso-PC at day 180 – 3.0

mol% or less); the lots with less than 80 ppm TEA accumulated lyso-PC at the rate of about 0.03 mg/mL/month, or less, and had less than 7 mol% of lyso-PC at day 180; the lot with 100 ppm residual TEA accumulated lyso-PC at the rate of about 0.06 mg/mL/month, and had about 10 mol% lyso-PC at day 180.

[00227] FIG. 7 is a graph showing the rates of lyso-PC accumulation (in mg/mL/month) stored at 5 ± 3 °C plotted against TEA content (in ppm) in the stabilized irinotecan sucrosofate liposome compositions, along with the linear regression line derived from the data. Five additional lots of liposomal irinotecan sucrosofate were prepared similarly to Example 3. The preparations were stored at irinotecan moiety (as explained above, based on the free base anhydrous) of about 4.3 mg/mL irinotecan free base anhydrous per mL and periodically analyzed for lyso-PC formation and TEA content as described in Example 3. The rates of lyso-PC accumulation were calculated as the slopes of linear regression lines obtained by fitting to the lyso-PC data over storage time for each lot and plotted against the TEA content with averaged TEA readings of the BDP/DP paired lots (FIG. 6). As follows from the graph, preparations that had about 25 ppm or less of TEA accumulated lyso-PC at the rates less than 0.02 mg/mL/month (less than 2.5 mol% lyso-PC increase over 180 day period); preparations that had less than about 70 ppm TEA accumulated lyso-PC at the rate of less than 0.033 mg/mL/month (less than 4.3 mol% lyso-PC increase over 180 day period), and all preparations had less than about 100 ppm of TEA and accumulated lyso-PC at the rate of less than 0.062 mg/mL/month (less than 8.0 mol% lyso-PC increase over 180 day period).

[00228] Samples 24, 25, and 28 each have less than 20 ppm (e.g., about 10-20 ppm) substituted ammonium ion (protonated TEA) and have the lowest amounts of lyso-PC observed after 6 months of refrigerated storage at 4 °C (2.2-3 mol% lyso-PC). Comparing samples 26 and 27, increasing the amount of residual substituted amine trapping agent counter-ion (e.g., protonated TEA) in the irinotecan SOS liposomes from about 39 ppm to 79 ppm (a 103% increase) was accompanied by an unexpected drop on the amount of lyso-PC observed after 180 days (from 6.9 mol% to 5.4 mol%, a 22% reduction in lyso-PC). However, further increasing the amount of residual substituted ammonium ion (e.g., protonated TEA) in the irinotecan SOS liposomes from 79 ppm (Sample 27) to 100 ppm (Sample 29) (i.e., a 27% increase) was accompanied by an additional 87% increase (i.e., from 5.4 mol% in Sample 27 to 10.1 mol% in Sample 29) in the amount of lyso-PC observed after 6 months of refrigerated storage at 4 °C.

Example 4: *Interaction of irinotecan with sucrosofate*

[00229] FIG. 8 is a graph showing gram-equivalent amounts of irinotecan and sucrosofate in the precipitate formed by combining irinotecan hydrochloride and triethylammonium sucrosofate in aqueous solution at various proportions of sucrosofate (SOS) as described in Example 4.

[00230] When a solution of irinotecan hydrochloride is combined with liposomes containing triethylammonium sucrosofate, a hydrogen ion can be scavenged and an irinotecan sucrosofate salt can be formed. To study the reaction between irinotecan and triethylammonium sucrosofate, we prepared 25 mM (16.93 mg/mL) aqueous solution of irinotecan hydrochloride trihydrate USP and 250 meq/L (31.25 mM) solution of triethylammonium sucrosofate (TEA-SOS) (essentially as described in the "Methods" section). Aliquots of irinotecan hydrochloride solution were diluted with water, heated to 65 °C, and combined with aliquots of TEA-SOS solution to produce a series of irinotecan-SOS gram-equivalent ratios between 9:1 and 1:9, at the overall gram-equivalent concentration of both compounds together equal 25 meq/L. The samples were quickly mixed by vortexing, incubated at 65 °C for 30 minutes, chilled in ice-water, and allowed to equilibrate overnight at 4-6 °C. In all samples, precipitation was observed. The next day, the samples were centrifuged at 10000xg for 5 minutes and at 14000xg for another 5 minutes, and clear supernatant fluid (over a loose, copious white to slightly tan precipitate) was isolated and analyzed for the amounts of non-precipitated irinotecan and SOS essentially as described in the Examples to determine the amount and composition of the precipitate. The results were plotted against the gram-equivalent percent of SOS in the sample (FIG. 8). In the range of 20-80 equivalent % of SOS the graphs for both components consisted of two linear branches that met at the value of 50 equivalent %, indicating that irinotecan and sucrosofate formed an insoluble salt with the stoichiometry of one irinotecan molecule per one sulfate ester group of sucrosofate (that is, eight molecules of irinotecan (IRI) per one sucrosofate (SOS) molecule):

$$8\ \mathrm{IRI.HCl} + \mathrm{TEA_8SOS} \rightarrow (\mathrm{IRI.H})_8\mathrm{SOS} \downarrow + \mathrm{TEACl}$$

[00231] Despite pronounced differences in the molecular size and shape of a protonated irinotecan molecule and a sucrosofate anion, their salt surprisingly kept close stoichiometry – eight molecules of protonated irinotecan for one sucrosofate molecule - even under the large excess of either component (FIG. 8). Thus, irinotecan sucrosofate can exist in the liposome in a poorly soluble, precipitated, or gelated form. The fact that the precipitating salt keeps its strict stoichiometry allows the process to advance to the point when mostly all or essentially all sulfate groups of sucrosofate are bound to the drug molecules. Consistent with the

irinotecan-sucrosofate gram-equivalent ratio measurements of Example 6, the process of irinotecan loading to obtain a stable liposome of the present invention, in some embodiments, can comprise liposomal precipitation of the stoichiometric drug salt until at least 90%, at least 95%, or even at least 98%, and in some cases, essentially all free liposomal sucrosofate is depleted from the liposomal aqueous phase through precipitation and/or gelation of its irinotecan salt.

Example 5: *Preparation and solubility determination of irinotecan sucrosofate.*

[00232] An amount of 1.64 g of irinotecan hydrochloride trihydrate was added to 160 mL of water acidified with 0.008 mL of 1 N HCl, and heated on a 65 °C water bath with stirring until the drug was dissolved. Five mL of 0.46 M (based on sulfate concentration) triethylammonium sucrosofate were added with intensive stirring, and stirred for five minutes more. A yellowish oily precipitate solidified into a brittle mass after overnight storage at 4-6 °C. The mass was triturated with a glass rod to give fluffy off-white precipitate and incubated under refrigeration for 25 days. The precipitate was separated by centrifugation, and the supernatant solution was discarded. The pellet was resuspended in five volumes of deionized water, and precipitated by centrifugation; this washing step was repeated two more times until the pH of the suspension was about 5.8. Finally, the pellet was resuspended in an equal volume of deionized water to give about 26 mL or the product, having an irinotecan content of 46.0 mg/mL (free base) (yield 84% of theory). An aliquot of the product was solubilized in 1 N HCl and analyzed for irinotecan (by spectrophotometry at 365 nm in 70% aqueous isopropanol-0.1 N HCl) and for sulfate after hydrolysis in a diluted (1:4) perchloric acid using a barium sulfate turbidimetric assay. The molar ratio of irinotecan to SO_4 was found to be 1.020 ± 0.011. Aliquots of the irinotecan sucrosofate suspension were added to deionized water to the final drug salt concentration of 0.93, 1.85, and 3.71 mg/mL. The samples were incubated with agitation at 4-6 °C for 22 hours, the solid material was removed by centrifugation for 10 min at 14000 g, and the supernatant fluid was analyzed for irinotecan by spectrophotometry. The concentration of irinotecan in solution was found to be 58.9 ± 0.90 micro-g/mL, 63.2 ± 0.6 micro-g/mL, and 63.4 ± 1.3 micro-g/mL, respectively, that, on average, corresponds to an irinotecan sucrosofate molar solubility of 1.32×10^{-5} M.

Example 6: *Various Irinotecan Liposomes*

[00233] All the experiments for this example were conducted using a 25mm extruder, hollow fibers, or tangential flow filtration (TFF) set-up for the initial diafiltration step, micro

scale drug loading, and a TFF set-up for the final diafiltration followed by EAV filtration. Due to the limited volume of the drug loaded material, the final filtration after dilution was done using a 20 cm^2 EAV filter in a biosafety cabinet instead of two EBV filters.

Table 11:

Sample	31a (2a)	31b (2b)	32a (3a)	32b (3b)	33 (4)	34 (5)
Encapsulated Irinotecan Concentration (mg/mL)	4.56	4.68	4.65	4.58	5.2	5.1
% Encapsulated Irinotecan (%)	98.4	99.2	98.2	99.3	99.7	99.8
DSPC: cholesterol mol ratio	3.03: 1.00		2.96: 1.00		3:1	3:1
Irinotecan:phospholipid ratio (mg/mol)	486	486	458	458	502	481
pH	7.28	7.28	6.41	6.41	7.3	7.3
Particle Size Measurement (USP729) (nm) (PDI)	90-130 (0.05)				110 (0.10)	109 (0.05)
Lyso PC Concentration (mg/mL)	<0.060	0.175	0.076	0.573	0.24	0.79
Lyso-PC concentration (mol%)[h]	4.04		12.72		5.11	16.43

[h] Measured according to Method A, as described herein.

[00234] Referring to Table 11, a series of different irinotecan liposomes were prepared having different amounts of lyso-PC. Unless otherwise indicated, the irinotecan liposomes encapsulated irinotecan sucrose octasulfate in a vesicle consisting of DSPC, cholesterol, and MPEG2000DSPE in a 3:2:0.015 mole ratio.

[00235] Sample 30 (lot 1) was obtained by preparing the liposomes as described in Example 1 (except as indicated in this Example) and then holding the extruded liposomes for 8 hours at 72 °C after liposome extrusion, pH adjusted to 6.2-6.9 at the end of 8 hours, resulting in a composition with about 45 mol% lyso-PC (i.e. about 1.7 mg/mL). The time of MLV preparation was considered as time 0. This experiment was performed using an aliquot from the baseline experiment 1. The composition of sample 30 (lot 1) was prepared with liposomes having a lower DSPC:cholesterol mol ratio (about 2:1 instead of 3:1 in other samples). The resulting irinotecan liposome composition had a high level of lyso-PC (i.e., greater than 1 mg/mL and greater than 40 mol% lyso-PC).

[00236] Samples 31a and 31b (lots 2a and 2b) were prepared using the process of Example 1 with modifications to test the effect of increasing the TEA-SOS solution concentration in the liposomes prior to irinotecan drug loading and the effect of decreasing the irinotecan drug

loading ratio by 15% on the characteristics of the resulting irinotecan liposome compositions. The material of sample 31a (2a) was obtained by forming liposomes having vesicles comprising DSPC and cholesterol (in the ratio provided in Table 11) encapsulating a solution of TEA-SOS at a 0.5 M sulfate group concentration to form multilamellar vesicles (MLVs) and contacting these liposomes with irinotecan hydrochloride solution in the amount of 510 g irinotecan free base anhydrous/mol of PL to load the drug into the liposomes. The material of sample 31b (2b) was obtained by maintaining the liposome composition of sample 31a (2a) for 1 week at 40 °C, then analyzing the sample again. The resulting irinotecan liposome compositions of samples 31a and 31 b (2a and 2b) both contained very low levels of lyso-PC (i.e. less than about 0.06 mg/mL or 4 mol% in sample 31a (2a) and about 0.175 mg/mL in sample 31b (2b)).

[00237] Samples 32a and 32b (lots 3a and 3b, respectively) were prepared using the process of Example 1, with modifications selected to study the combined effect of formulation buffer pH and decreased irinotecan drug loading ratio. The material of sample 32a (3a) was obtained by forming liposomes having vesicles comprising DSPC and cholesterol (in the ratio provided in Table 10) encapsulating a solution of TEA-SOS solution to form MLVs and contacting these liposomes with irinotecan to load the drug into the liposomes, forming irinotecan sucrose octasulfate within the liposome at the irinotecan drug loading ratio indicated in Table 11 (lower irinotecan drug loading ratio than samples 33 (4) and 34 (5)) in a buffer selected to provide a pH of about 6.50 (instead of a pH of about 7.25 in sample 30 (1)). The material of sample 32b (3b) was obtained by maintaining the composition of sample 3a for 1 week at 40 °C, then analyzing the sample again. The resulting irinotecan liposome compositions 32a(3a) and 32b (3b) both contained low levels of 0.076 mg/mL and 0.573 mg/mL lyso-PC, respectively.

[00238] Samples 33 (4) and 34 (5) were prepared according to the methods described in Example 1. The material of sample 33 (4) and 34 (5) was obtained by forming liposomes having vesicles comprising DSPC and cholesterol (in the ratio provided in Table 11) encapsulating a solution of TEA-SOS solution to form MLVs and contacting these liposomes with irinotecan to load the drug into the liposomes, forming irinotecan sucrose octasulfate within the liposome at 500 g irinotecan moiety (based on the free base anhydrous)/mol phospholipid in a buffer selected to provide a pH of about 7.25 (instead of a pH of about 6.5 in samples 3a and 3b). The resulting irinotecan liposome compositions 3a and 3b both contained low levels of 0.24 mg/mL and 0.79 mg/mL lyso-PC, respectively.

[00239] FIG. 12 is a graph showing the amount of lyso-PC measured in sample 33(4) (circles, lower line) and sample 34(5) ("+" data points, upper line). The rate of lyso-PC formation was higher in Sample 34 (5) than Sample 33 (4). The linear fit to the data points in FIG. 12 was as follows:

Sample 33 (4): lyso-PC, mg/mL = 0.0513596 + 0.0084714*Accumulated Age

Sample 34 (5): lyso-PC, mg/mL = 0.1766736 + 0.0279783*Accumulated Age

The total lyso-PC concentration of the irinotecan liposome preparations in Samples 33 and 34 were 0.24 mg/mL and 0.79 mg/mL at 22 months, respectively.

Example 7: *Irinotecan Liposome Injection (ONIVYDE®)*

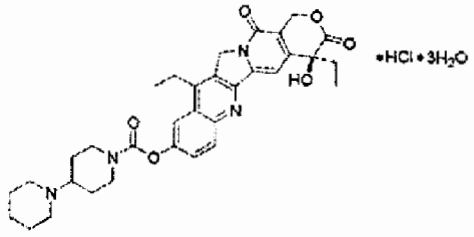
[00240] One preferred example of a storage stable irinotecan liposome preparation is the product marketed as ONIVYDE® (irinotecan liposome injection) *(Merrimack Pharmaceuticals, Inc., Cambridge, MA)*. The ONIVYDE® product is a topoisomerase inhibitor, formulated with irinotecan hydrochloride trihydrate into a liposomal dispersion, for intravenous use. The ONIVYDE® product is indicated, in combination with fluorouracil and leucovorin, for the treatment of patients with metastatic adenocarcinoma of the pancreas after disease progression following gemcitabine-based therapy.

[00241] The recommended dose of the ONIVYDE® product is 70 mg/m^2 administered by intravenous infusion over 90 minutes once every 2 weeks. The ONIVYDE® product is administered in combination with leucovorin and fluorouracil for the treatment of certain forms of pancreatic cancer. The recommended starting dose of the ONIVYDE® product in these pancreatic cancer patients known to be homozygous for the UGT1A1*28 allele is 50 mg/m^2 administered by intravenous infusion over 90 minutes. Increase the dose of the ONIVYDE® product to 70 mg/m^2 as tolerated in subsequent cycles. There is no recommended dose of the ONIVYDE® product for patients with serum bilirubin above the upper limit of normal.

[00242] The ONIVYDE® product is administered to patients as follows. First, the calculated volume of the ONIVYDE® product is withdrawn from the vial. This amount of the ONIVYDE® product is then diluted in 500 mL 5% Dextrose Injection, USP or 0.9% Sodium Chloride Injection, USP and mixed by gentle inversion. The dilution should be protected from light. The dilution is then administered within 4 hours of preparation when stored at room temperature or within 24 hours of preparation when stored under refrigerated conditions [2 °C to 8 °C (36 °F to 46 °F)]. The diluted solution is allowed to come to room temperature

prior to administration, and it should not be frozen. The dilution is then infused over 90 minutes without the use of in-line filters, and the unused portion is discarded.

[00243] The ONIVYDE® product is formulated with irinotecan hydrochloride trihydrate, a topoisomerase inhibitor, into a liposomal dispersion for intravenous use. The chemical name of irinotecan hydrochloride trihydrate is (S)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1H-pyrano[3',4':6,7]-indolizino[1,2-b]quinolin-9-yl-[1,4'bipiperidine]-1'-carboxylate, monohydrochloride, trihydrate. The empirical formula is $C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3H2O$ and the molecular weight is 677.19 g/mole. The molecular structure is:



[00244] The ONIVYDE® product is provided as a sterile, white to slightly yellow opaque isotonic liposomal dispersion. Each 10 mL single-dose vial contains the equivalent of 43 mg irinotecan free base at a concentration of 4.3 mg/mL irinotecan free base anhydrous per mL (i.e., 4.3 mg irinotecan moiety/mL). The liposome is a unilamellar lipid bilayer vesicle, approximately 110 nm in diameter, which encapsulates an aqueous space containing irinotecan in a gelated or precipitated state as the sucrose octasulfate salt. The vesicle is composed of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) 6.81 mg/mL, cholesterol 2.22 mg/mL, and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE) 0.12 mg/mL. Each mL also contains 2-[4-(2-hydroxyethyl) piperazin-1-yl]ethanesulfonic acid (HEPES) as a buffer 4.05 mg/mL and sodium chloride as an isotonicity reagent 8.42 mg/mL.

[00245] Irinotecan liposome injection is a topoisomerase 1 inhibitor encapsulated in a lipid bilayer vesicle or liposome. Topoisomerase 1 relieves torsional strain in DNA by inducing single-strand breaks. Irinotecan and its active metabolite SN-38 bind reversibly to the topoisomerase 1-DNA complex and prevent re-ligation of the single-strand breaks, leading to exposure time-dependent double-strand DNA damage and cell death. In mice bearing human tumor xenografts, irinotecan liposome administered at irinotecan HCl-equivalent doses 5-fold lower than irinotecan HCl achieved similar intratumoral exposure of SN-38.

[00246] The plasma pharmacokinetics of total irinotecan and total SN-38 were evaluated in patients with cancer who received the ONIVYDE® product, as a single agent or as part of

combination chemotherapy, at doses between 50 and 155 mg/m2, and 353 patients with cancer using population pharmacokinetic analysis.

[00247] The pharmacokinetic parameters of total irinotecan and total SN-38 following the administration of the ONIVYDE® product at 70 mg/m2 as a single agent or part of combination chemotherapy are presented below.

Table 12: *Summary of Mean (±Standard Deviation) Total Irinotecan and Total SN-38*

Dose (mg/m^2)	Total Irinotecan					Total SN-38		
	C_{max} [µg/mL] (n=25)	$AUC_{0-\infty}$ [h·µg/mL] (n=23)	$t_{1/2}$ [h] (n=23)	CL [L/h] (n=23)	V_d [L] (n=23)	C_{max} [ng/mL] (n=25)	$AUC_{0-\infty}$ [h·ng/mL] (n=13)	$t_{1/2}$ [h] (n=13)
70	37.2 (8.8)	1364 (1048)	25.8 (15.7)	0.20 (0.17)	4.1 (1.5)	5.4 (3.4)	620 (329)	67.8 (44.5)

C_{max}: Maximum plasma concentration
$AUC_{0-\infty}$: Area under the plasma concentration curve extrapolated to time infinity
$t_{1/2}$: Terminal elimination half-life
CL: Clearance
V_d: Volume of distribution

[00248] Over the dose range of 50 to 155 mg/m2, the Cmax and AUC of total irinotecan increases with dose. Additionally, the Cmax of total SN-38 increases proportionally with dose; however, the AUC of total SN-38 increases less than proportionally with dose.

[00249] Direct measurement of irinotecan liposome showed that 95% of irinotecan remains liposome-encapsulated, and the ratios between total and encapsulated forms did not change with time from 0 to 169.5 hours post-dose.

[00250] The ONIVYDE® product should be stored at 2 °C to 8 °C (36 °F to 46 °F), should be protected from light, and should not be frozen.

[00251] Multiple ONIVYDE® product preparations were placed on long term stability and analyzed over 12-36 months of storage at 2-8 °C (refrigerated conditions). Results are plotted in graphs in FIG. 9, 10, 11A, and 11B, as described below. In one study, the particle size (FIG. 9) and Particle Size Distribution (FIG. 10) were measured for 12 ONIVYDE® product preparations over 12-36 months. The PDI remained well below 0.1, and below about 0.05, for all samples. In another study, the pH (FIG. 11A) was measured for 13 different ONIVYDE® product preparations over 12 -36 months. The pH remained above 6.8 during the study for all samples. In another study, the amount of lyso-PC (FIG. 11B) was measured over 12 months for 16 different ONIVYDE® product preparations, during refrigerated storage. The amount of lyso-PC remained below 1 mg/mL for all samples.

[00252] For the purpose of determining the irinotecan free base concentration in the ONIVYDE® product embodiment at different time points of storage, irinotecan free base is quantified as provided in the "Example" section. For the purpose of determining the lipid

composition of the ONIVYDE® product embodiment at different time points of storage, lipids are quantified using standard HPLC methodologies that are standard in the art.

[00253] For the purpose of determining the mean particle size (D) and polydispersity index (PDI) of liposomes of the ONIVYDE® product embodiment at different time points of storage, the DLS method in conjunction with a Malvern ZetaSizer Nano ZS™ was used.

[00254] For the purpose of determining the presence of lyso-PC in the ONIVYDE® product embodiment at different time points of storage, lyso-PC is quantified as described in the "Examples" section. Additionally, it is also contemplated within the context of the present invention that lyso-PC may be quantified by HPLC as described in the specification.

Example 8: *Topotecan and Vinorelbine Liposomes*

[00255] The aim of this storage stability study was to determine any changes in the physical and chemical stability of topotecan (TPT) liposomes and vinorelbine (VNB) liposomes prepared with a sucrose octasulfate trapping agent, when stored at 4 °C. Specifically, the study examined whether, during liposome manufacture, reducing the sucrose octasulfate (SOS) trapping agent concentration from 0.6 M to 0.45 M sulfate groups, while maintaining topotecan or vinorelbine to phospholipid ratio as indicated below per mol phospholipid, would have an effect on the amount of lyso-PC present in the liposome samples. Similarly, the effect of increases in the pH from 6.5 to 7.5 was examined, to determine whether this pH increase reduced the presence of lyso-PC in the liposome compositions. TPT and VNB were encapsulated with a SOS trapping agent in liposomes containing DSPC, cholesterol (Chol), and PEG-DSPE in a 3:2:0.015 molar ratio. The formulation parameters investigated include: solution pH (6.5-7.5), concentration of the sucrose octasulfate trapping agent during liposome preparation (0.45-0.6 M sulfate), the drug encapsulated (TPT or VNB), and the drug to lipid ratio (500 g TPT HCl per mol phospholipid during liposome loading; for VNB, from 350 to 450 g VNB moiety per mol phospholipid during liposome loading). The various physicochemical properties of the liposomes that were monitored during this stability study were: liposome size, drug to phospholipid ratio, drug encapsulation efficiency, general appearance, and lyso-lipid formation.

[00256] DSPC, cholesterol (Chol), and PEG-DSPE were weighed out in amounts that corresponded to a 3:2:0.015 molar ratio, respectively (790.15 mg/257.8 mg/14.0 mg). The lipids were dissolved in chloroform/methanol (4/1, v/v), mixed thoroughly, and divided into 2 aliquots (A and B). Each sample was evaporated to dryness using a rotary evaporator at 60 °C. Residual chloroform was removed from the lipids by placing under vacuum (180 µtorr) at

room temperature for 12 hours. The dried lipids were dissolved in ethanol at 60 °C, and pre-warmed TEA_8SOS of appropriate concentration was added so that the final alcohol content was 10% (v/v). The total phospholipid concentration was approximately 75 mM. The lipid solution was extruded through 0.1 μm polycarbonate membranes (Nuclepore™) 10 times, to produce liposomes with a typical average diameter of 95-115 nm. The pH of the extruded liposomes was adjusted as needed (with 1 N NaOH) to pH 6.5 if necessary. The liposomes were purified by a combination of ion-exchange chromatography and size-exclusion chromatography. First, Dowex™ 1RA 910 resin was treated with 1 N NaOH, followed by 3 washes with deionized water, and then followed by 3 washes of 3 N HCl, and then multiple washes with water. The conductivity of the eluted fractions was measured by using a flow-cell conductivity meter (Pharmacia, Uppsala, Sweden). The fractions were deemed acceptable for further purification if the conductivity was less than 15 μS/cm. The liposome eluate was then applied to a Sephadex G-75 (Pharmacia) column equilibrated with deionized water, and the collected liposome fraction was measured for conductivity (typically less than 1 μS/cm). 40% dextrose solution was added to achieve a final concentration of 5% (w/w), and the buffer (Hepes) was added from a stock solution (0.5 M, pH 6.5) to a final concentration of 10 mM.

[00257] A stock solution of topotecan hydrochloride was prepared by dissolving 50 mg in 10 mL deionized water. Drugs were added to liposome solutions at the drug/lipid ratio indicated for each formulation in the results Table 13. For TPT loading, the pH was adjusted to pH 6.0 prior to loading. Vinorelbine was added directly from the commercial USP injection solution from the pharmacy, and the pH of the resulting mixture adjusted to 6.5 with 1 N NaOH prior to heating. Drug loading was initiated by heating the liposome/drug mixtures to 60 °C for 30 minutes. The solutions were rapidly cooled upon removal from the water bath by immersing in ice cold water. Extra liposomal drug was removed by size exclusion chromatography, using Sephadex G75 columns equilibrated and eluted with Hepes (10 mL) buffered saline (HBS), pH 6.5. The samples were analyzed for irinotecan by HPLC and phosphate by the method of Bartlett (see Phosphate Determination).

[00258] For storage, the samples were divided into 4 mL aliquots, and the pH was adjusted if necessary using 1 N HCl or 1 N NaOH, sterile filtered under aseptic conditions, and filled into sterile clear glass vials that were sealed under argon with a Teflon® lined threaded cap and placed in a thermostatically controlled refrigerator at 4 °C. At defined time points, an aliquot was removed from each sample and tested for appearance, size, drug/lipid ratio, and drug and lipid chemical stability. The liposome size was determined in the diluted samples by

dynamic light scattering using Coulter Nano-Sizer at 90 degree angle and presented as Mean ± Standard deviation (nm) obtained by the method of cumulants.

[00259] The results from comparative stability studies are provided in Table 13.

Table 13: *Topotecan and Vinorelbine Liposomes prepared with TEA_8SOS Trapping Agent (0.6N SOS sulfate groups, stored at 2 mg/mL drug concentration)*

Sample	Drug	[gram of drug]/ total mol PL	pH	Time (months)	Mol % Lyso-PC[i]	Size±SD
19	TPT	500[j]	6.5	0	0	115.0±9.5
				1	12.2(±0.71)	107.3±16.9
				3	25.0(±0.9)	108.4±9.1
				6	25.9(±0.5)	102.3±25.2
				9	29.0(±1.4)	108.6±19.2
20	TPT	500[j]	7.25	0	0	115.0±9.5
				1	10.0(±0.4)	109.0±16.8
				3	19.0(±0.5)	108.6±15.8
				6	23.3(±2.2)	105.5±13.6
				9	29.4(±3.1)	110.6±12.1
21	VNB	350	6.5	0	0	115.0±9.5
				1	2.2(±1.1)	105.3±16.7
				3		105.8±18.1
				6	9.5(±1.2)	102.8±8.9
				9	9.5 (±0.6)	103.4±23.3
22	VNB	350	7.25	0	0	115.0±9.5
				1	1.3(±0.1)	105.3±16.7
				3		105.8±18.1
				6	5.0 (±0.5)	102.8±8.9
				9	5.5 (±2.6)	103.4±23.2
23	VNB	450	6.5	0	0	115.0±9.5
				1	0.3(±0.1)	90.6±29.6
				3		104.7±21.2
				6	3.1(±1.1)	106.4±16.7
				9	3.4(±0.3)	133.3±16.6

[i] Measured according to Method B, as described herein.

[j] 500 g topotecan HCl per mol total phospholipids

[00260] The effect of storage media pH on the production of lyso-lipid in topotecan loaded liposomes was not observed in Samples 19 and 20. Both formulations in samples 19 and 20 exhibited close to 30 mol% lyso-lipid after 9 months, even though sample 19 was stored at pH 6.5 and sample 20 was stored at pH 7.25.

[00261] In contrast to both the liposomal camptothecins, liposomal vinorelbine was more resistant to lipid hydrolysis, in that the highest amount of lyso-lipid measured was in sample 21, having 9.5 mol% lyso-lipid after 9 months. Although less pronounced, we can also detect a dependence on the Stability Ratio and storage media pH. Higher Stability Ratio resulted in

reduced lipid hydrolysis (compare samples 21 to 23). A pH of 7.25 also reduced the amount of observed lipid hydrolysis (compare samples 21 to 22).

Example 9: HPLC Method for Measuring Lyso-PC (*"Method A")*

[00262] The amount of lyso-PC in the irinotecan sucrose octasulfate liposome preparations tested to obtain data in FIGS. 11B and 12 was obtained using HPLC with detection by evaporative light scattering. A suitable HPLC method (referred herein to "Method A") is a quantitative method used to measure the amount of stearic acid, lyso-PC, cholesterol, and DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine) in the drug product. The liposomes are dissociated into their individual lipid components using a methanol-tetrahydrofuran solution. The lipid components are quantitated using reverse phase high pressure liquid chromatography equipped with an evaporative light scattering detector.

Sample and Standard Preparation

Standard Preparation:

LysoPC

[00263] A five point standard curve is prepared by diluting appropriate quantities of LysoPC with 85:15 methanol-tetrahydrofuran to target final concentrations of 4, 8, 20, 32, and 40 µg/mL.

Stearic Acid

[00264] A five point standard curve is prepared by diluting appropriate quantities of stearic acid with 85:15 methanol-tetrahydrofuran to target final concentrations of 2, 4, 10, 16, and 20.4 µg/mL.

Cholesterol

[00265] A five point standard curve is prepared by diluting appropriate quantities of cholesterol with 85:15 methanol-tetrahydrofuran to target final concentrations of 90, 144, 183.7, 224.9 and 266.6 µg/mL.

DSPC

[00266] A five point standard curve is prepared by diluting appropriate quantities of DSPC with 85:15 methanol-tetrahydrofuran to target final concentrations of 220, 352, 449, 549.8, and 651.7 µg/mL.

Assay Control

[00267] An assay control is prepared by diluting stearic acid in diluent (85:15 methanol-tetrahydrofuran) to a target final concentration of 9.0 µg/mL and 18.0 µg/mL.

Sample Preparation:

[00268] Samples are prepared by diluting each sample in 85:15 methanol-tetrahydrofuran solution to a target final DSPC concentration of 475 µg/mL.

Solution Stability

[00269] The test samples standards, and assay controls have demonstrated acceptable stability in solution for up to 48 hours when stored at ambient temperature.

Instrument and Instrument Parameters

[00270] A suitable high pressure chromatographic system equipped with an evaporative light scattering detector capable of changing gain and filter settings throughout a run, if need be, to ensure proper peak detection. The instrument operating parameters are listed in Table 14.

Table 14: *Chromatographic Conditions*

Chromatographic Parameter	**Chromatographic Conditions and Set Points**		
Column	Phenomenex Luna C8(2) 100 µm, 150 mm x 3 mm with guard column Phenomenex C8 4x2.0mm		
Injection Volume	20 µL		
Column Temperature	30°C		
Flow Rate	1.0 mL/minutes		
Mobile Phase A	100 mM Ammonium Acetate pH 4.0		
Mobile Phase B	Methanol		
ELSD Settings	Gas Pressure: 3.5 bar Temperature: 40°C		
Gradient	**Time (minutes)**	**Mobile Phase A (%)**	**Mobile Phase A (%)**
	0	15	85
	3	8	92
	6	0	100
	9	0	100
	9.1	15	85
	12	15	85

Table 15: *System Suitability*

Parameter	Acceptance Criteria
Elution Profile	Chromatographic profile of diluent blank, working standard, and assay control are comparable to the examples shown in the test method.
Plates	Average plates ≥2000 for DSPC and Cholesterol in calibration standard level 5 (n=5 injections)
Tailing	Average tailing ≤1.5 for DSPC and Cholesterol in calibration standard level 5 (n=5 injections)
Signal- to-noise	Signal-to-noise ≥10 for LysoPC peak in calibration standard level 1
Precision	%RSD ≤6.0 for LysoPC, stearic acid, DSPC and cholesterol in in calibration standard level 5 (n=5 injections)
Linearity	R^2 ≥0.99 for LysoPC, stearic acid, DSPC and cholesterol standard calibration curves.
Accuracy	%Recovery = 90 – 110% for DSPC and cholesterol within standard calibration curves
Accuracy	%Recovery = 80-120% for stearic acid control

[00271] Each lipid concentration is determined by analyzing the sample peak area to the standard curve. A second order polynomial equation (quadratic curve) trend line is used to calculate the lipid concentrations of lyso-PC and Stearic Acid. A linear trend line is used to calculate the lipid concentrations of DSPC and cholesterol.

[00272] A representative chromatogram is presented in FIG. 13A and FIG. 13B.

[00273] All references cited herein are incorporated herein by reference in their entirety.

CLAIMS

1. A storage stabilized liposomal irinotecan composition having a pH of 7.00-7.50 and comprising a dispersion of irinotecan liposomes encapsulating irinotecan sucrose octasulfate in vesicles consisting of cholesterol and the phospholipids 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE), at a concentration of irinotecan moiety equivalent to, in grams of irinotecan free anhydrous base, 500 mg (±10%) irinotecan moiety per mmol total liposome phospholipid and 4.3 mg irinotecan moiety per mL of the liposomal irinotecan composition, the storage stabilized liposomal irinotecan composition stabilized to form less than 20 mol% Lyso-PC during the first 6 months of storage at 4 °C.

2. A storage stabilized liposomal irinotecan composition having a pH of 7.00-7.50 and comprising a dispersion of irinotecan liposomes encapsulating irinotecan sucrose octasulfate in unilamellar bilayer vesicles consisting of cholesterol and the phospholipids 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE), at a concentration of irinotecan moiety equivalent to, in grams of irinotecan free anhydrous base, 500 mg (±10%) irinotecan moiety per mmol total liposome phospholipid and 4.3 mg irinotecan moiety per mL of the liposomal irinotecan composition, the storage stabilized liposomal irinotecan composition having an irinotecan/sulfate compound gram-equivalent ratio of 0.85-1.2.

3. A storage stabilized liposomal irinotecan composition stabilized to form less than 20 mol% Lyso-PC during the first 6 months of storage at 4 °C, the liposomal irinotecan composition made by a process comprising steps of:

(a) forming a lipid dispersion in a solution made from TEA_8SOS and/or DEA_8SOS having a sulfate concentration of from 0.4 to 0.5 M and a pH between from 5 to 7, the lipids in said dispersion being DSPC, cholesterol and MPEG-2000-DSPE in an about 3:2:0.015, respectively, mole ratio;

(b) extruding the lipid dispersion between 60-70 °C through at least one 0.1 μm membrane to form liposomes;

(c) substantially removing ions derived from TEA_8SOS and/or DEA_8SOS that are outside the liposomes;

(d) contacting the liposomes at a temperature between 60-70 °C with a solution made using irinotecan free base or irinotecan salt, thereby forming a preparation of liposomes encapsulating irinotecan;

(e) substantially removing substances derived from the TEA_8SOS and/or DEA_8SOS and irinotcan ingredients that are outside the liposomes; and

(f) adjusting the pH of the composition to be from 7.0 to 7.5.

4. The liposomal irinotecan composition of any one of claims 1-3, made by a process comprising steps of:

(a) forming a lipid dispersion in a solution made from TEA_8SOS having a sulfate concentration of from 0.4 to 0.5 M and a pH between from 5 to 7, the lipids in said dispersion being DSPC, cholesterol and MPEG-2000-DSPE in an about 3:2:0.015, respectively, mole ratio;

(b) extruding the lipid dispersion between 60-70 °C through at least one 0.1 μm membrane to form liposomes;

(c) substantially removing ions derived from TEA_8SOS that are outside the liposomes;

(d) contacting the liposomes at a temperature between 60-70 °C with a solution made using irinotecan free base or irinotecan salt, thereby forming a preparation of liposomes encapsulating irinotecan;

(e) substantially removing substances derived from the TEA_8SOS and irinotcan ingredients that are outside the liposomes; and

(f) adjusting the pH of the composition to be from 7.0 to 7.5.

5. The liposomal irinotecan composition of claim 4, wherein the lipid dispersion is extruded through at least two stacked 0.1 μm polycarbonate membranes.

6. The liposomal irinotecan composition of any one of the previous claims, where the liposomes have a mean size of 110 nm as determined by dynamic light scattering and where the size is determined by the method of cumulants.

7. The liposomal irinotecan composition of any one of the previous claims, having a total irinotecan moiety content equivalent to of 4.3 mg/ml irinotecan free base anhydrous.

8. The liposomal irinotecan composition of any one of claim 3-6, wherein:
in step (a) the liposomes are formed from TEA_8SOS having a sulfate concentration of between 0.43-0.47 M; and
in step (d) the solution made using irinotecan free base or an irinotecan salt has an irinotecan moiety content equivalent to 500 g (±10%) of irinotecan free base anhydrous per mole of DSPC; and
in step (f) adjusting the pH of the composition to be from 7.2 to 7.3.

9. The liposomal composition of any one of the previous claims, containing less than 1 mol% lyso-phosphatidylcholine (lyso-PC) prior to storage at about 4 °C, and 20 mol% or less (with respect to total liposome phospholipid) of lyso-PC after 180 days of storage at about 4 °C.

10. The liposomal composition of claim 9, containing 20 mol% or less (with respect to total liposome phospholipid) of lyso-phosphatidylcholine (lyso-PC) after 6, 9 or 12 months of storage at about 4 °C.

11. The liposomal irinotecan composition of any one of the previous claims, comprising a total of 6.1 to 7.5 mg DSPC/ml, 2 to 2.4 mg cholesterol /ml, and 0.11 to 0.13 mg MPEG-2000-DSPE/ml, all in an aqueous isotonic buffer.

12. The liposomal irinotecan composition of any one of the previous claims, wherein the liposomal irinotecan comprises the irinotecan liposomes in an isotonic HEPES aqueous buffer at a concentration of between 2 and 20 mM.

13. The liposomal irinotecan composition of any one of the previous claims, further comprising sodium chloride at a concentration of from 130-160 mM.

14. The liposomal irinotecan composition of any one of the previous claims, wherein the irinotecan encapsulated in the liposomes is in a gelated or precipitated state as a sucrose octasulfate salt.

15. The liposomal irinotecan composition of any one of the previous claims, wherein the irinotecan liposomes have a diameter of 95-115 nm, as measured by quasi-elastic light scattering.

16. The liposomal irinotecan composition of any one of the previous claims, comprising a total of 6.81 mg DSPC/ml, 2.22 mg cholesterol /ml, and 0.12 mg MPEG-2000-DSPE/ml, 4.05 mg/mL HEPES aqueous buffer and 8.42 mg sodium chloride/mL.

17. The liposomal irinotecan composition of any one of the previous claims, having a pH of 7.25, wherein the irinotecan liposomes have a diameter of 110 nm as measured by quasi-elastic light scattering.

18. The liposomal irinotecan composition of any one of the previous claims, forming less than 1 mg/mL lyso-phosphatidylcholine (lyso-PC) after 6 months of storage at about 4 °C.

19. The liposomal irinotecan composition of any one of the previous claims, made by a process comprising steps of:

(a) forming a lipid dispersion in a solution of TEA_8SOS having a sulfate concentration of about 0.45 M and a pH of about 6.5, the lipids in said dispersion consisting of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE) in a mole ratio of 3:2:0.015, respectively;

(b) extruding the lipid dispersion between 60-70 °C through at least one 0.1 μm membrane to form liposomes;

(c) removing ions derived from TEA_8SOS that are outside the liposomes;

(d) contacting the liposomes at a temperature between 60-70 °C with a solution made using irinotecan hydrochloride trihydrate, to form a preparation of liposomes encapsulating about 500 g (±10%) irinotecan per mol total liposome phospholipid;

(e) removing substances derived from the TEA_8SOS and irinotcan ingredients that are outside the liposomes; and

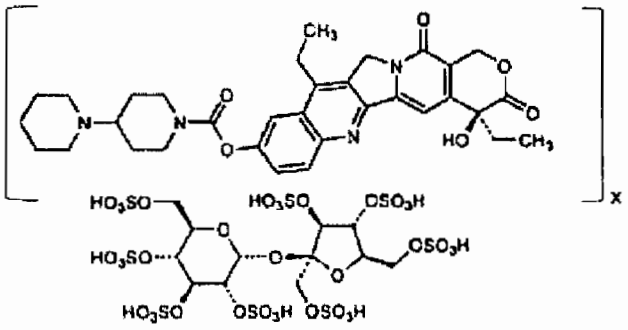
(f) adjusting the pH of the composition to be about 7.3.

20. The liposomal irinotecan composition of any of the previous claims, comprising a total of less than 100 ppm of TEA.

21. The liposomal irinotecan composition of any one of the previous claims, comprising a total of 30-100 ppm of TEA or DEA.

22. The liposomal irinotecan composition of any one of the previous claims, wherein at least 98% of the irinotecan is encapsulated in the irinotecan liposomes after 6 months of storage at about 4 °C.

23. The liposomal irinotecan composition of any one of the previous claims, comprising the irinotecan composition of formula (I) within the irinotecan liposomes, where x is 8:



(I).

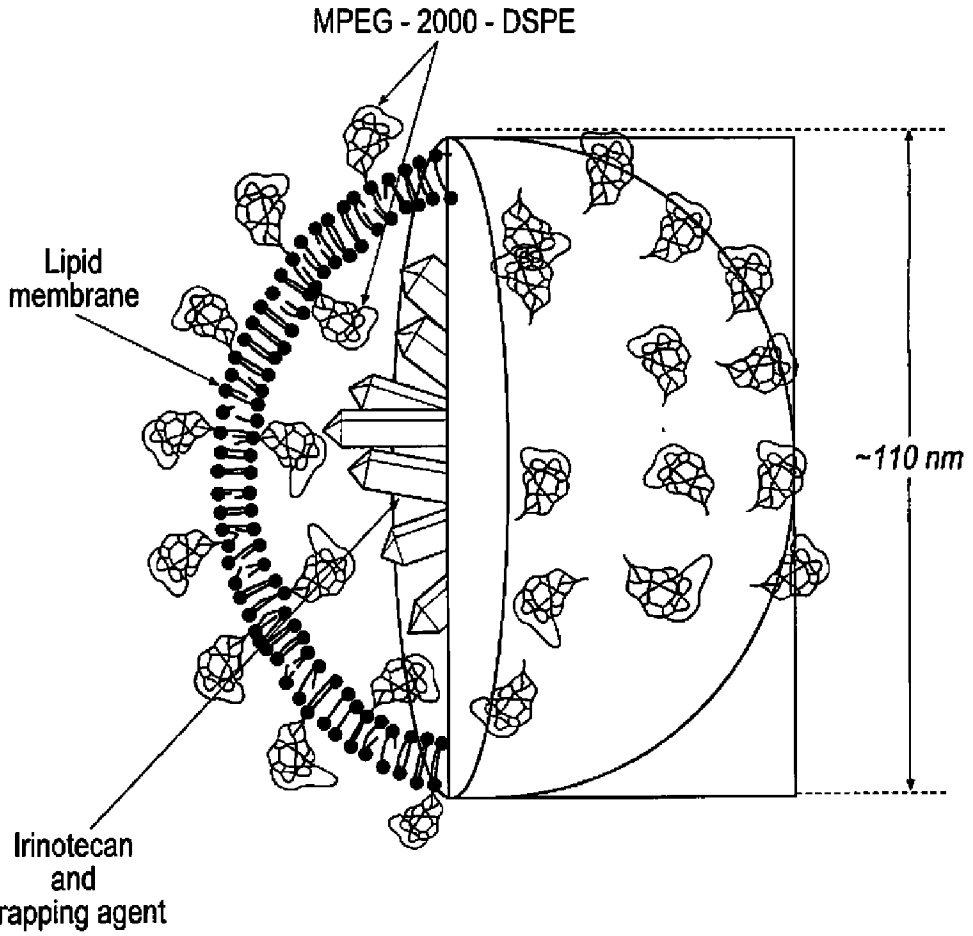

MPEG - 2000 - DSPE
Lipid membrane
~110 nm
Irinotecan and Trapping agent

FIG. 1A

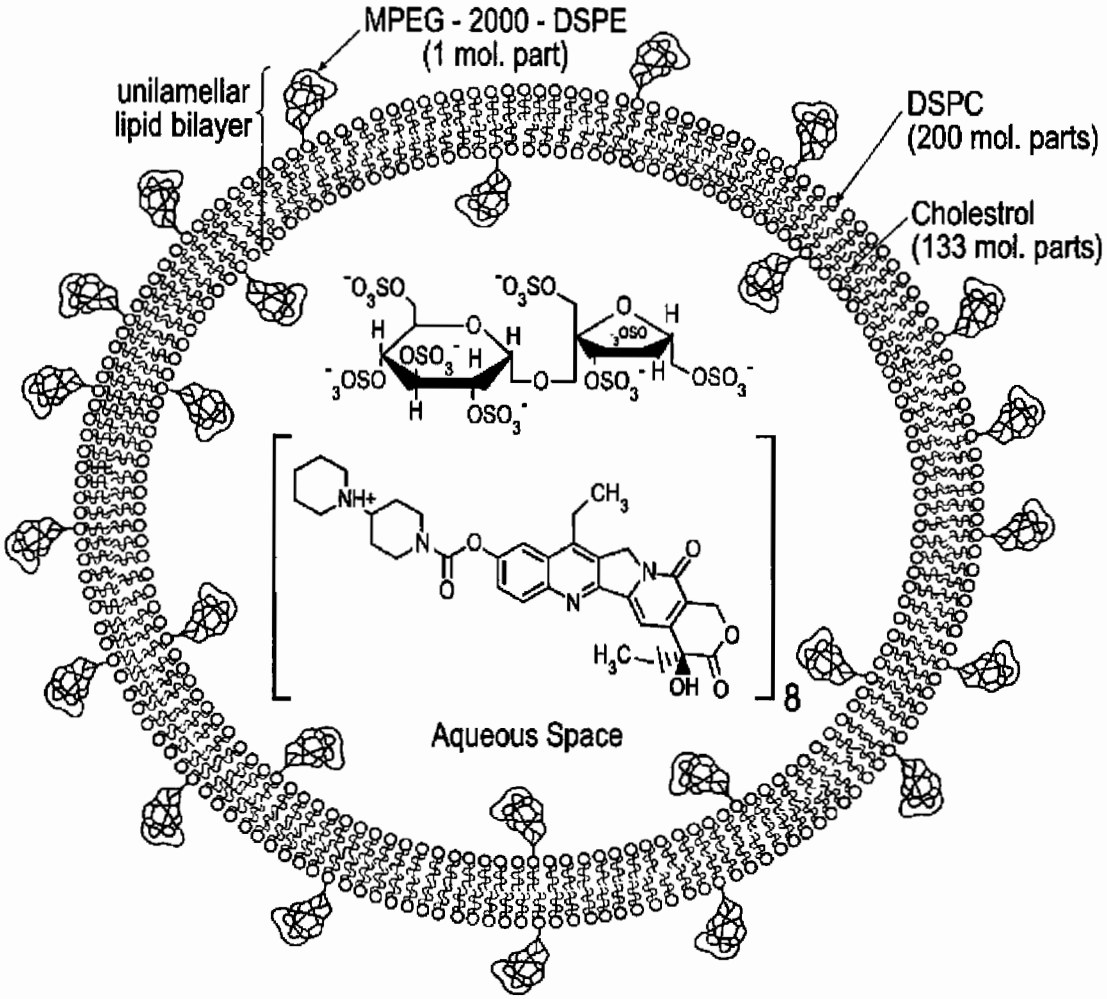

MPEG - 2000 - DSPE
(1 mol. part)
unilamellar
lipid bilayer
DSPC
(200 mol. parts)
Cholestrol
(133 mol. parts)
Aqueous Space
8

FIG. 1B

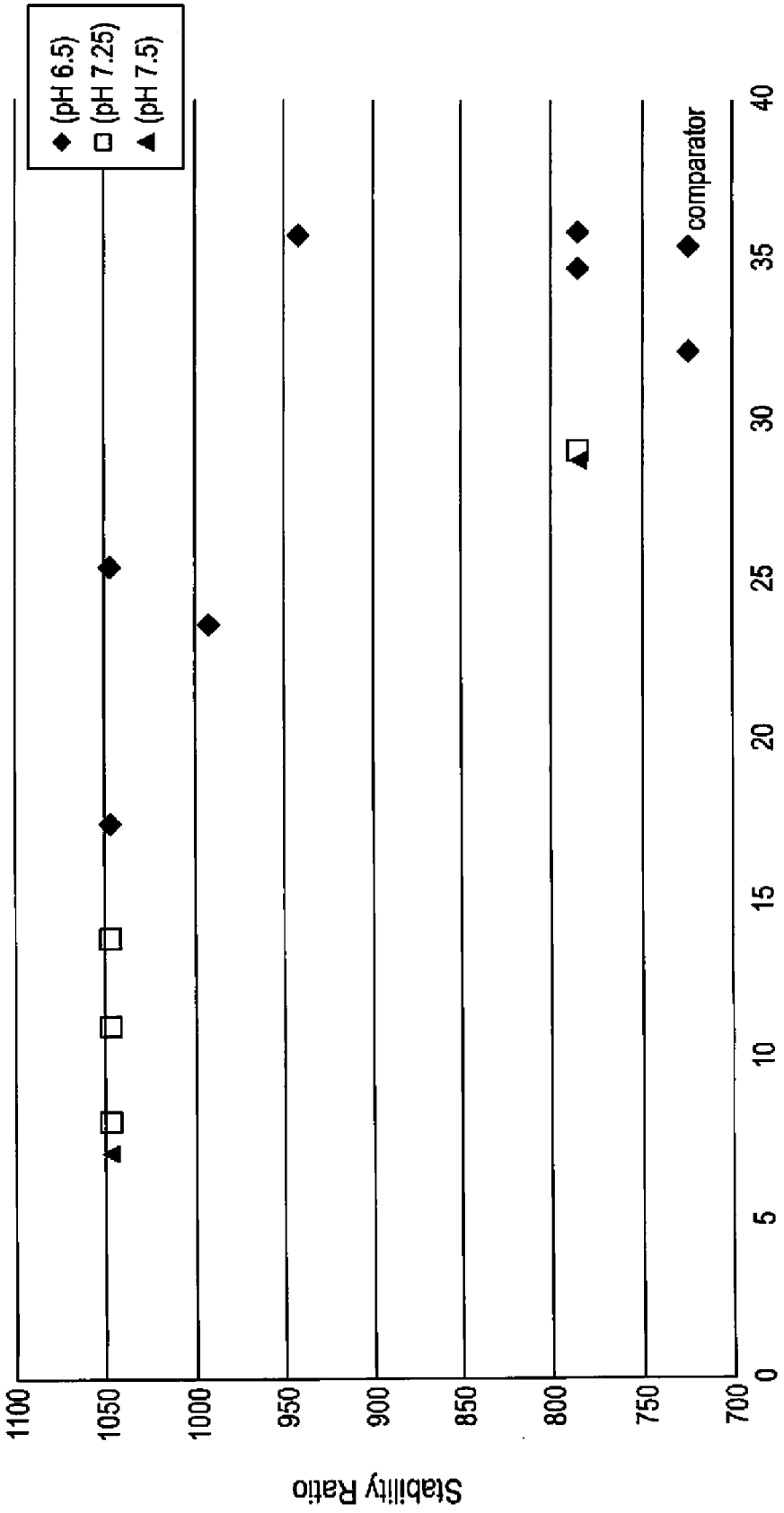

(pH 6.5)
(pH 7.25)
(pH 7.5)
comparator
Stability Ratio
1100
1050
1000
950
900
850
800
750
700
0
5
10
15
20
25
30
35
40
mol% liposomal Lyso-PC (9 mos, 4 °C)

FIG. 2A

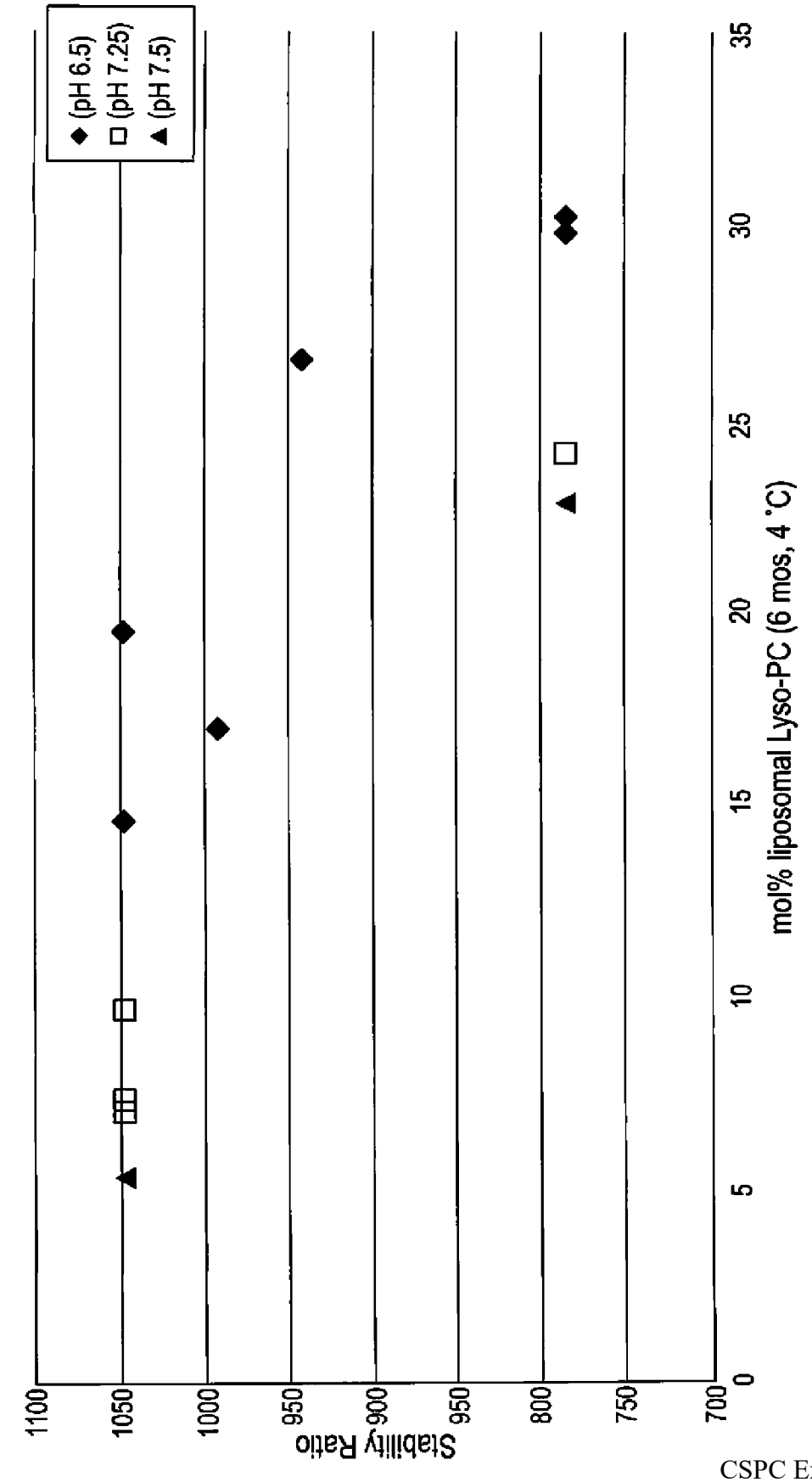

(pH 6.5)
(pH 7.25)
(pH 7.5)
Stability Ratio
1100
1050
1000
950
900
950
800
750
700
0
5
10
15
20
25
30
35
mol% liposomal Lyso-PC (6 mos, 4 °C)

FIG. 2B

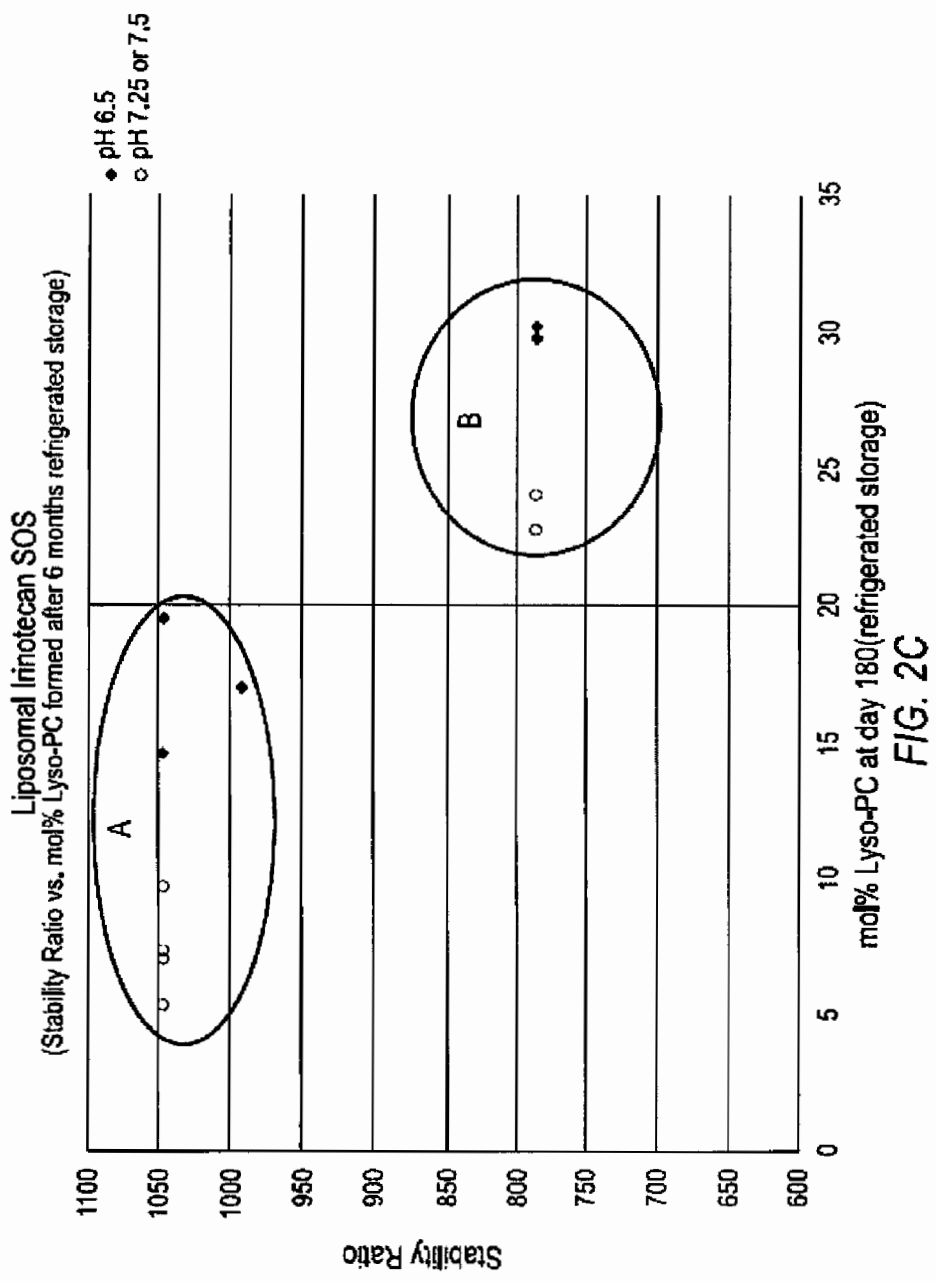

Liposomal Irinotecan SOS
(Stability Ratio vs. mol% Lyso-PC formed after 6 months refrigerated storage)
◆ pH 6.5
○ pH 7.25 or 7.5
A
B
Stability Ratio
1100
1050
1000
950
900
850
800
750
700
650
600
0
5
10
15
20
25
30
35
mol% Lyso-PC at day 180(refrigerated storage)

FIG. 2C

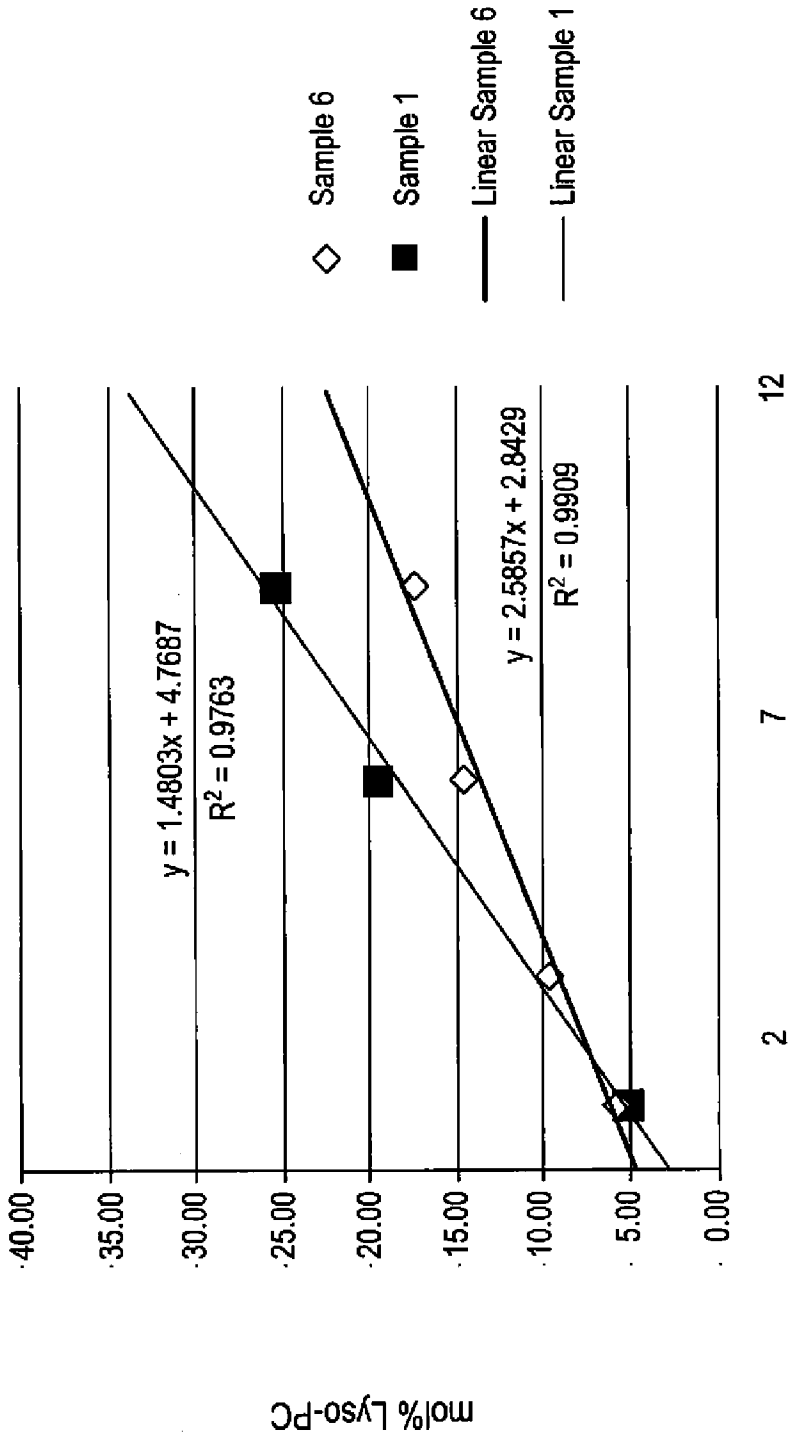

Sample 6
Sample 1
Linear Sample 6
Linear Sample 1
y = 1.4803x + 4.7687
R² = 0.9763
y = 2.5857x + 2.8429
R² = 0.9909
mol% Lyso-PC
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
2
7
12
months storage (4 °C)

FIG. 3A

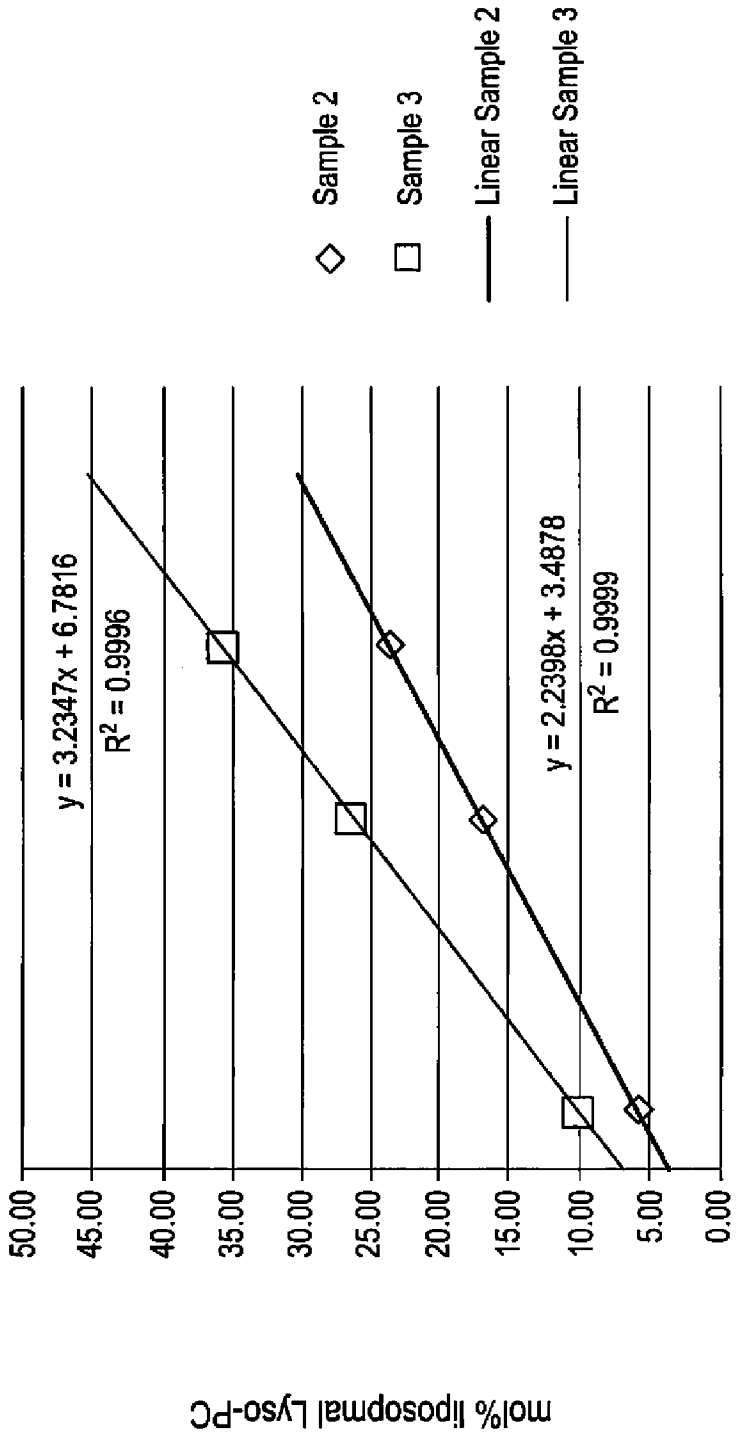

Sample 2
Sample 3
Linear Sample 2
Linear Sample 3
y = 3.2347x + 6.7816
R² = 0.9996
y = 2.2398x + 3.4878
R² = 0.9999
mol% liposomal Lyso-PC
months storage (4 °C)
50.00
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
2
7
12

FIG. 3B

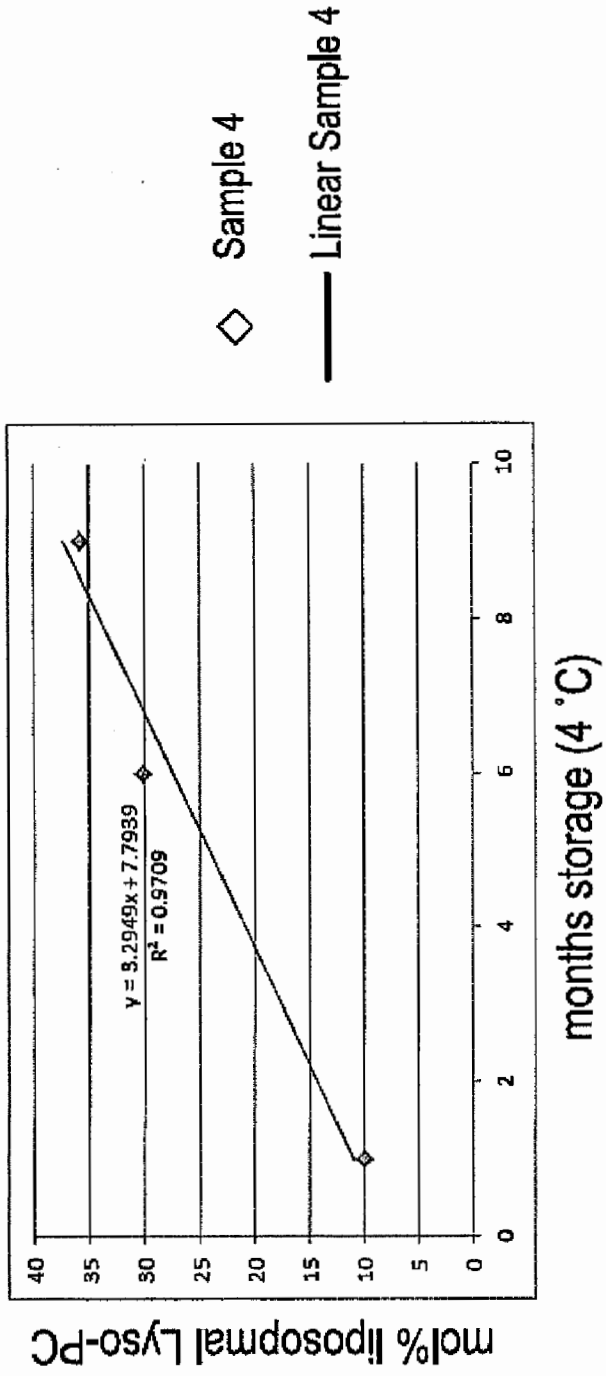

Sample 4
Linear Sample 4
mol% liposomal Lyso-PC
months storage (4 °C)
y = 3.2949x + 7.7939
R² = 0.9709
0
5
10
15
20
25
30
35
40
0
2
4
6
8
10

FIG. 3C

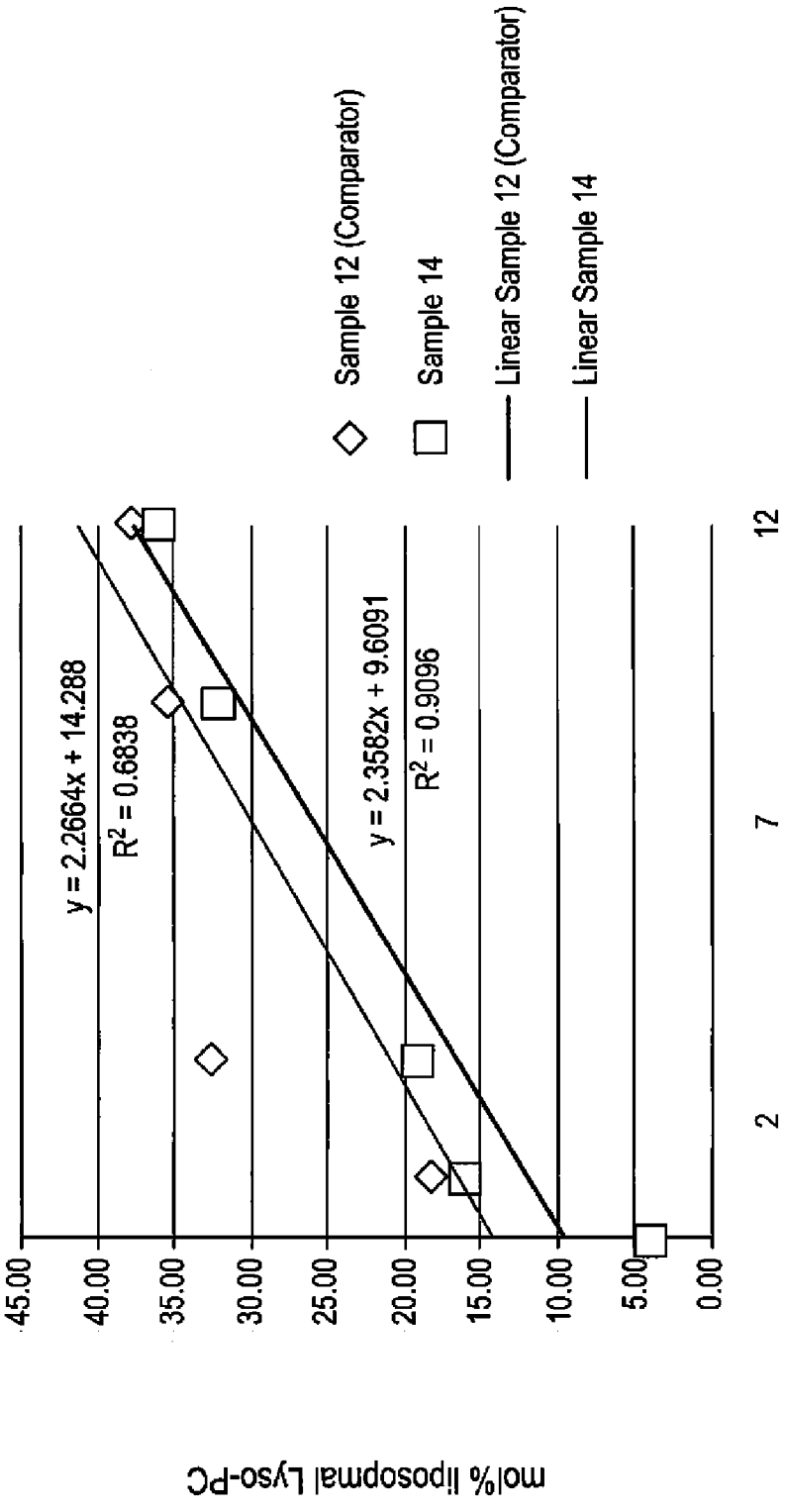

Sample 12 (Comparator)
Sample 14
Linear Sample 12 (Comparator)
Linear Sample 14
y = 2.2664x + 14.288
R² = 0.6838
y = 2.3582x + 9.6091
R² = 0.9096
mol% liposomal Lyso-PC
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
2
7
12
months storage (4 °C)

FIG. 3D

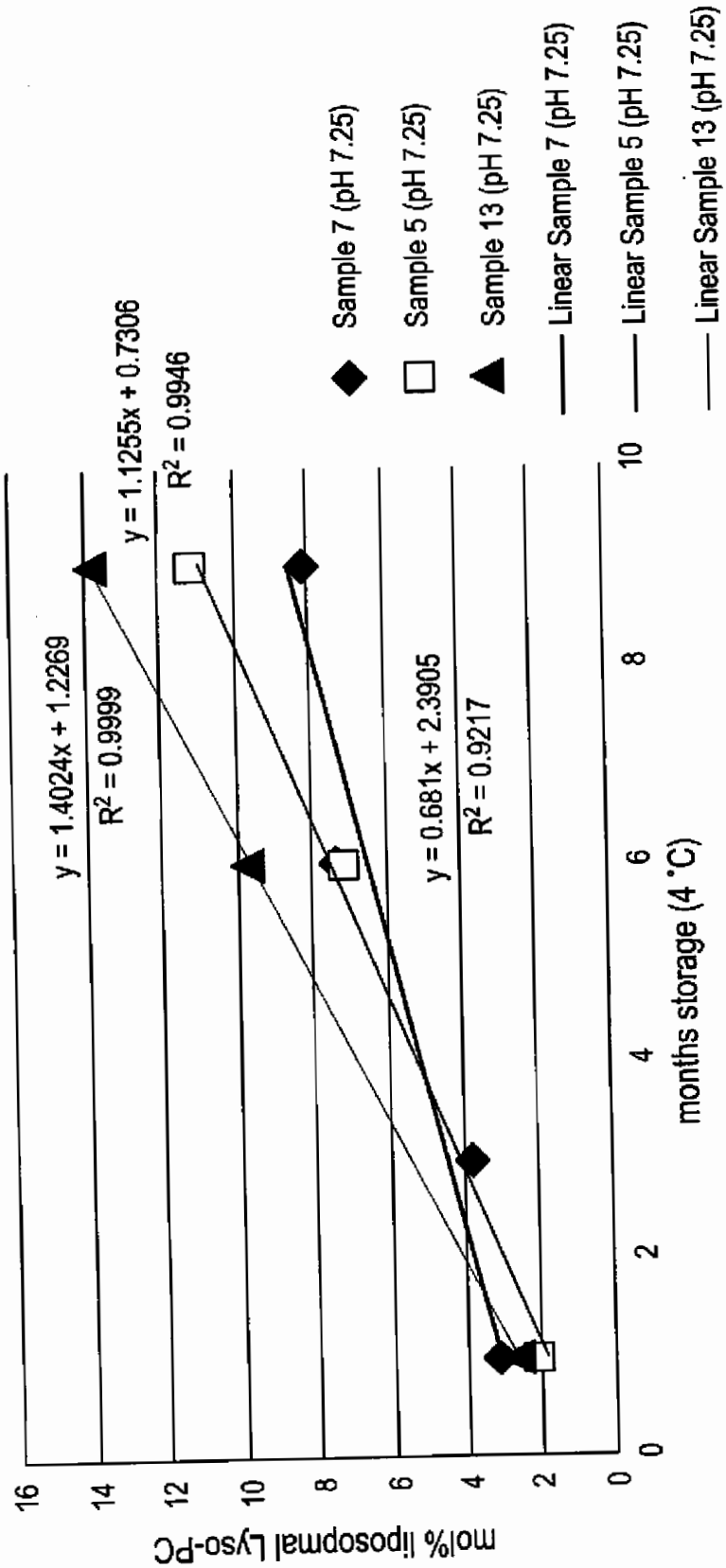

mol% liposomal Lyso-PC
months storage (4 °C)
y = 1.4024x + 1.2269
R² = 0.9999
y = 1.1255x + 0.7306
R² = 0.9946
y = 0.681x + 2.3905
R² = 0.9217
Sample 7 (pH 7.25)
Sample 5 (pH 7.25)
Sample 13 (pH 7.25)
Linear Sample 7 (pH 7.25)
Linear Sample 5 (pH 7.25)
Linear Sample 13 (pH 7.25)

FIG. 4A

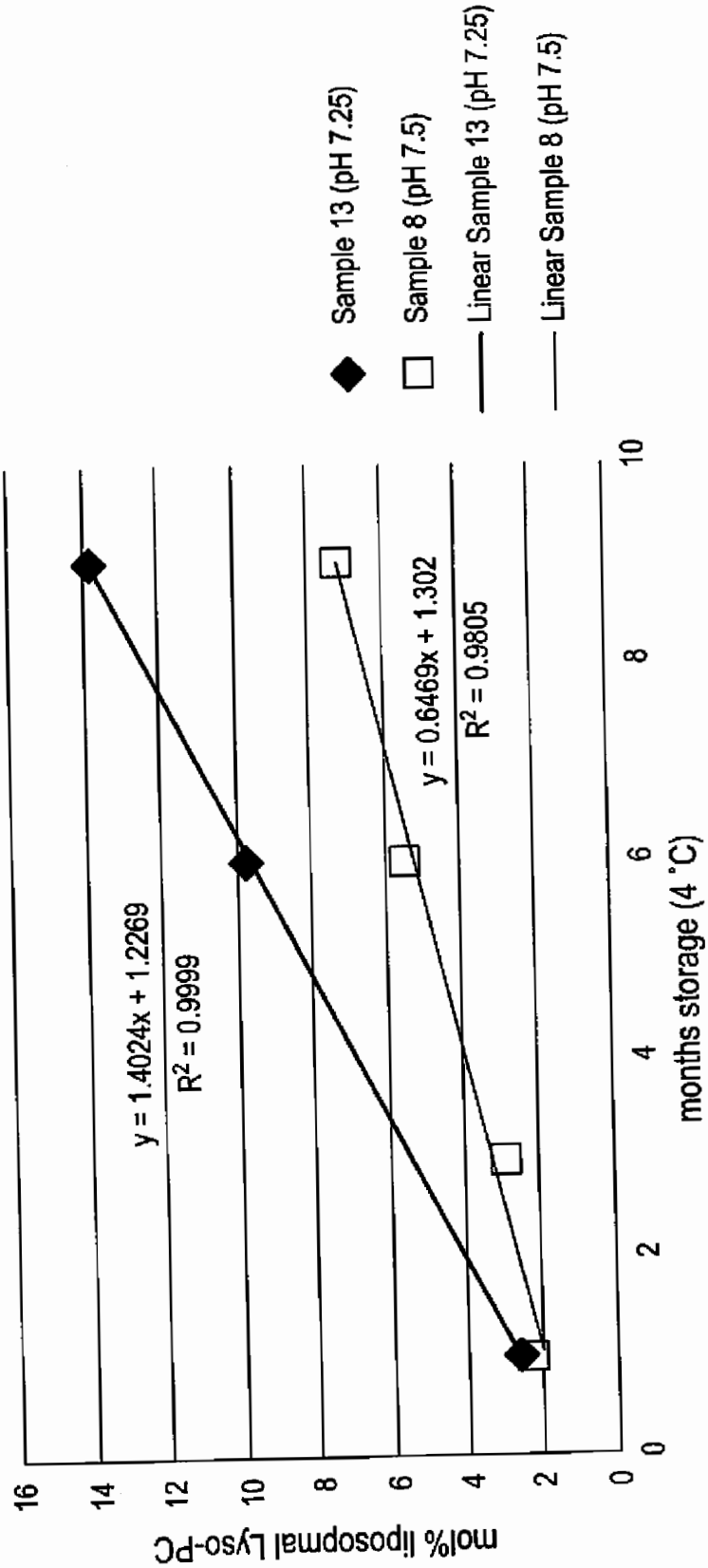

Sample 13 (pH 7.25)
Sample 8 (pH 7.5)
Linear Sample 13 (pH 7.25)
Linear Sample 8 (pH 7.5)
y = 1.4024x + 1.2269
R² = 0.9999
y = 0.6469x + 1.302
R² = 0.9805
mol% liposomal Lyso-PC
months storage (4 °C)
0
2
4
6
8
10
12
14
16

FIG. 4B

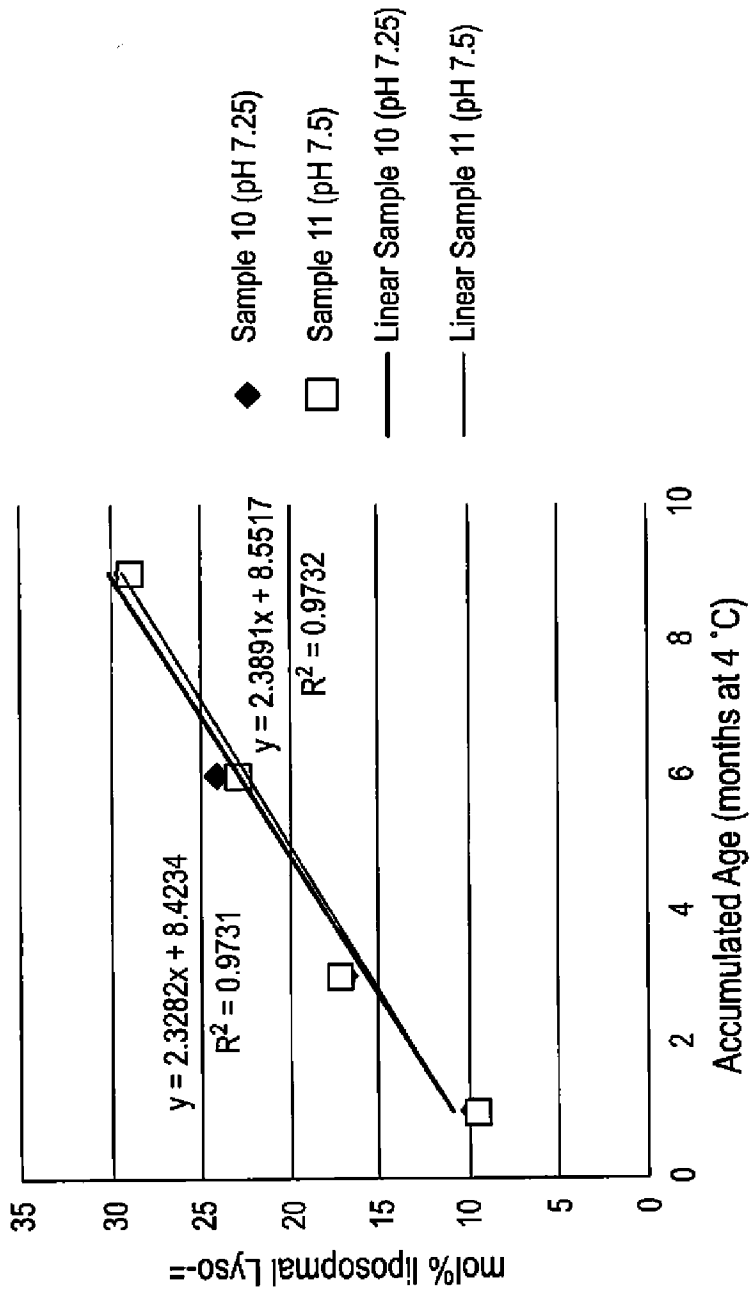

Sample 10 (pH 7.25)
Sample 11 (pH 7.5)
Linear Sample 10 (pH 7.25)
Linear Sample 11 (pH 7.5)
y = 2.3282x + 8.4234
R² = 0.9731
y = 2.3891x + 8.5517
R² = 0.9732
mol% liposomal Lyso-PC
Accumulated Age (months at 4 °C)
0
5
10
15
20
25
30
35
0
2
4
6
8
10

FIG. 4C

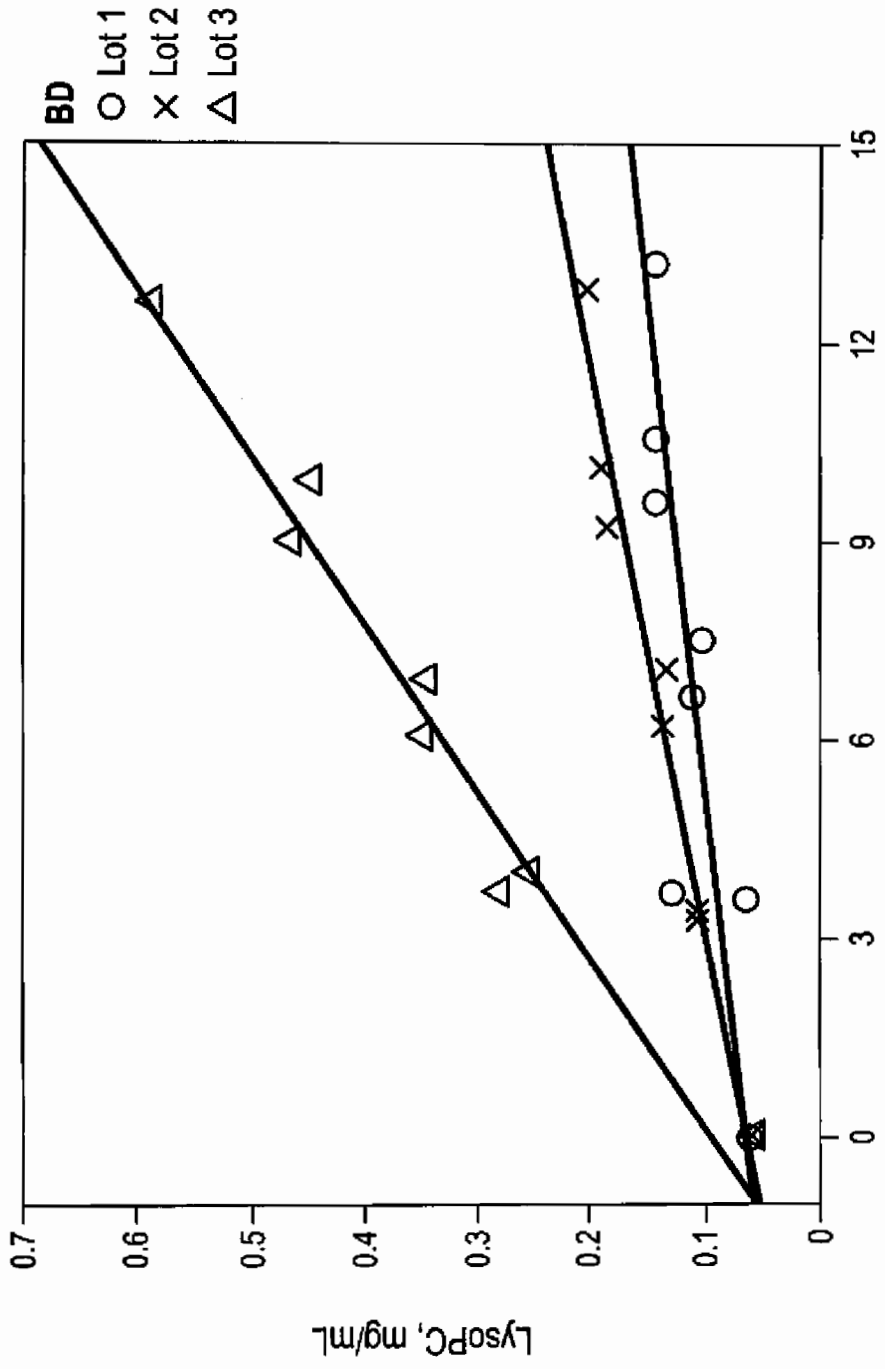

BD
O Lot 1
× Lot 2
Δ Lot 3
LysoPC, mg/mL
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
3
6
9
12
15
Accumulated Age (months at 4 °C)

FIG. 5

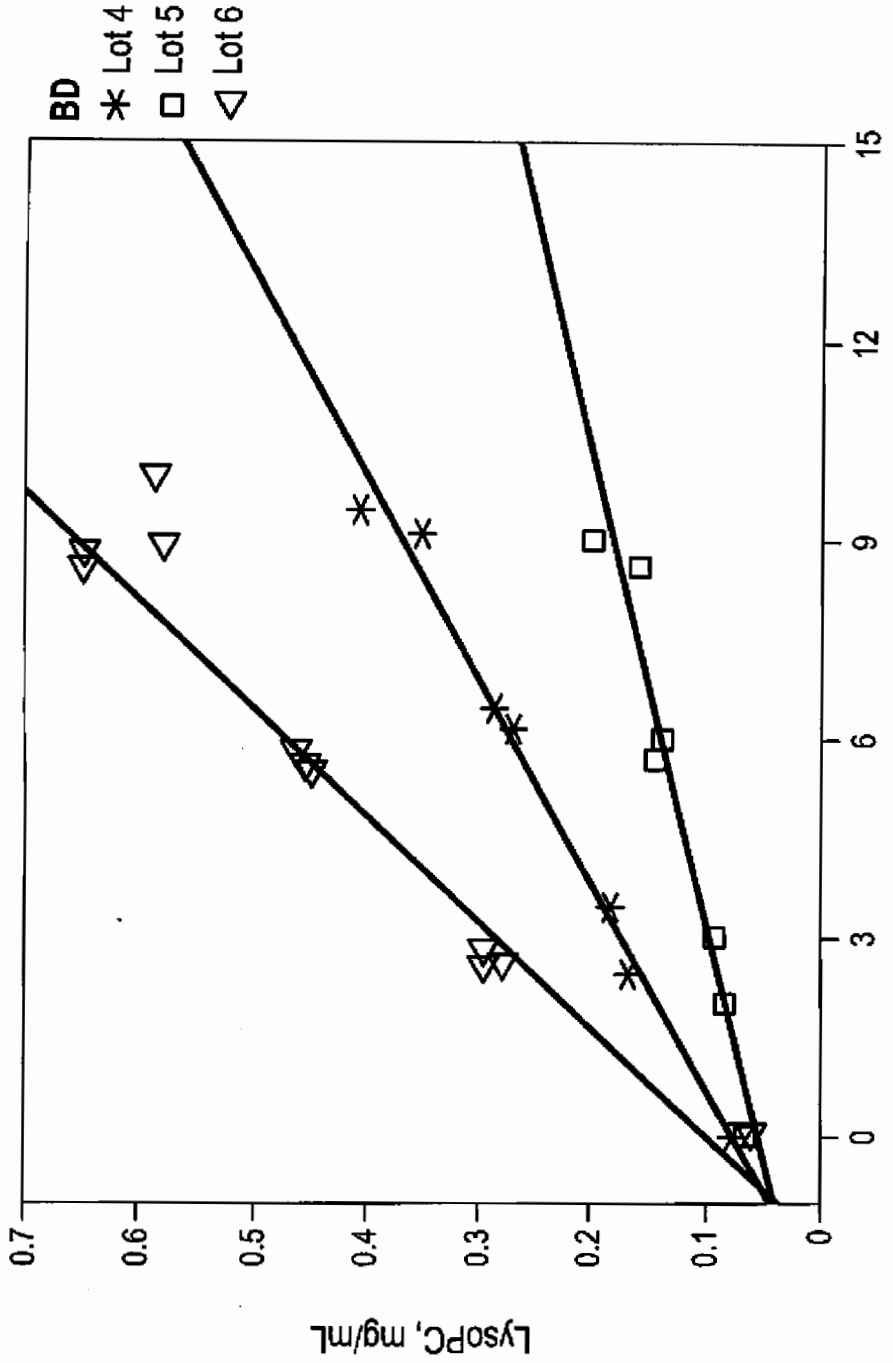

BD
Lot 4
Lot 5
Lot 6
LysoPC, mg/mL
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
3
6
9
12
15
Accumulated Age (months at 4 °C)

FIG. 6

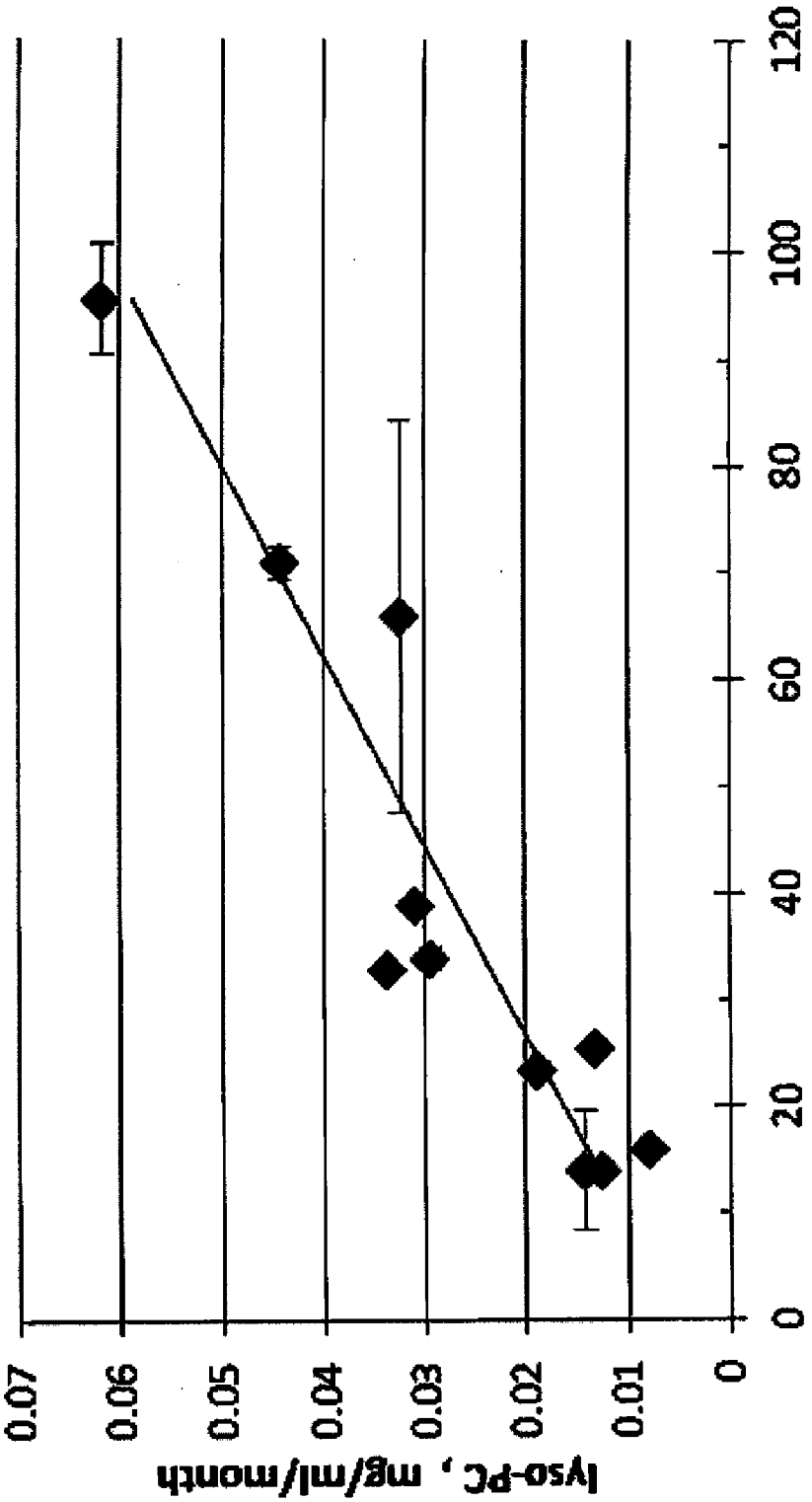

0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
lyso-PC , mg/ml/month
0
20
40
60
80
100
120
TEA, ppm

FIG. 7

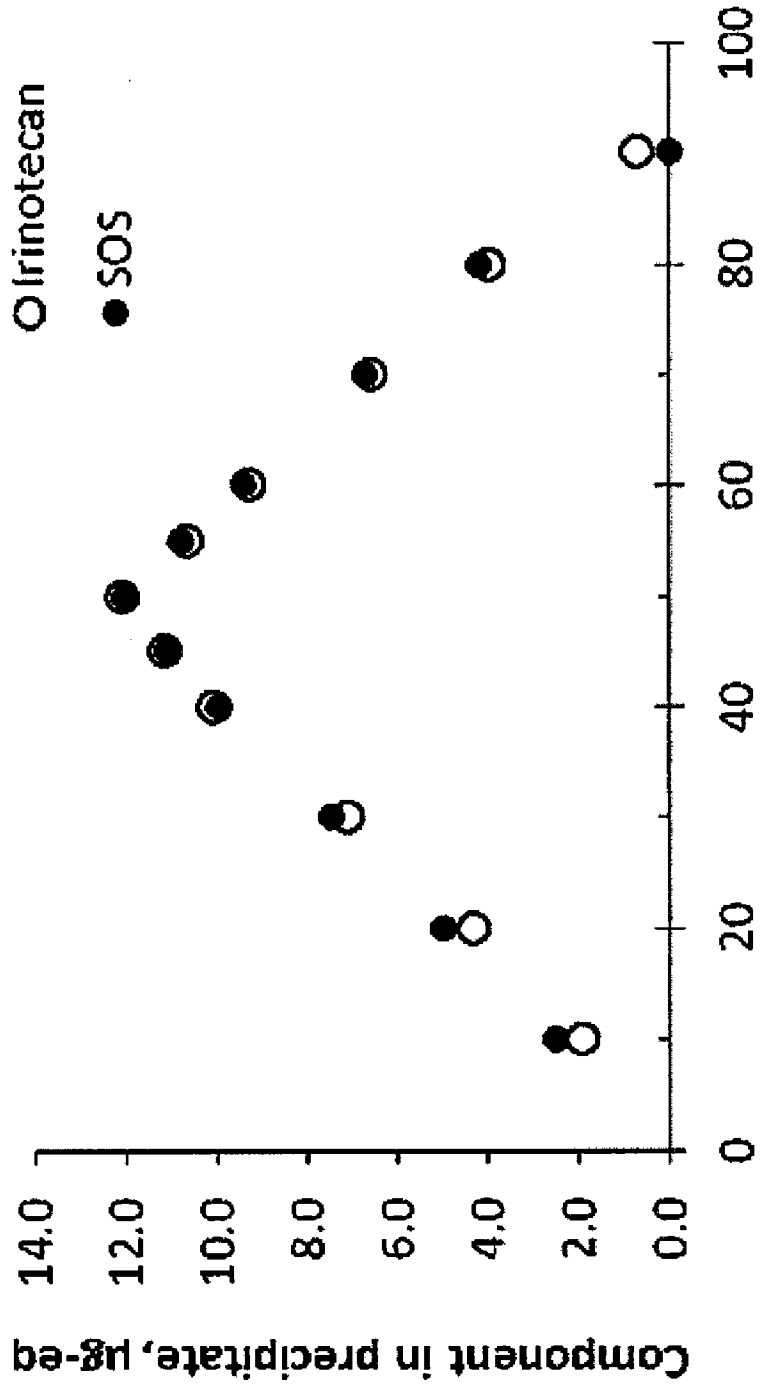

Irinotecan
SOS
Component in precipitate, µg-eq
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0
20
40
60
80
100
SOS, eq. %

FIG. 8

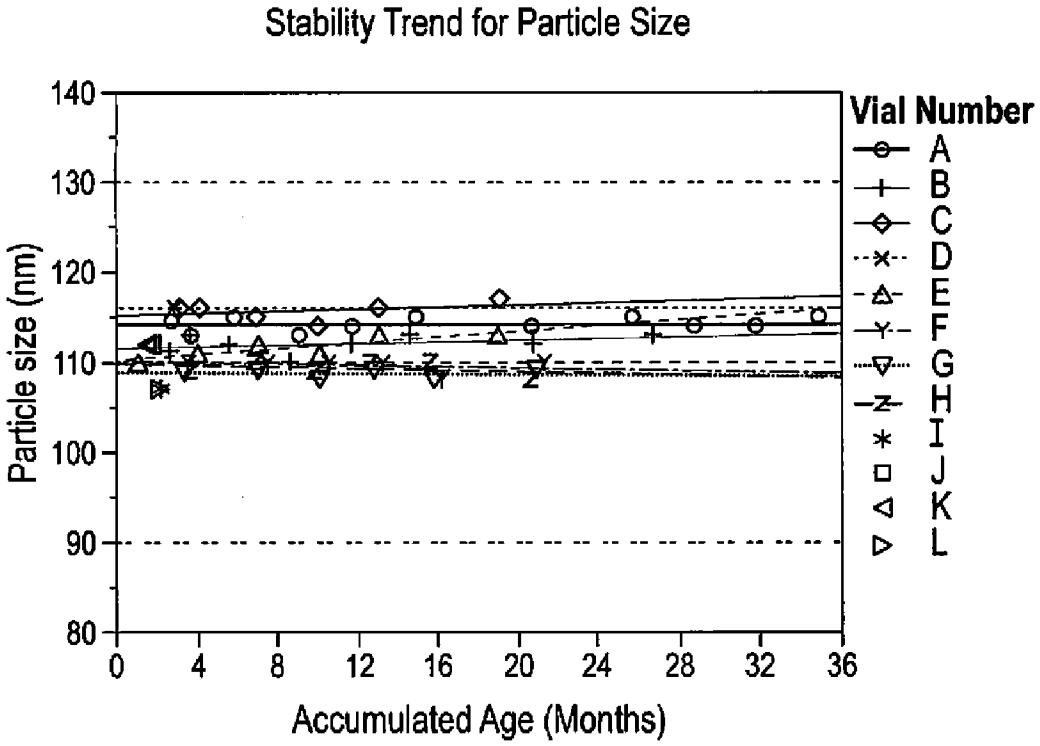

Stability Trend for Particle Size
Particle size (nm)
140
130
120
110
100
90
80
0
4
8
12
16
20
24
28
32
36
Accumulated Age (Months)
Vial Number
A
B
C
D
E
F
G
H
I
J
K
L

FIG. 9

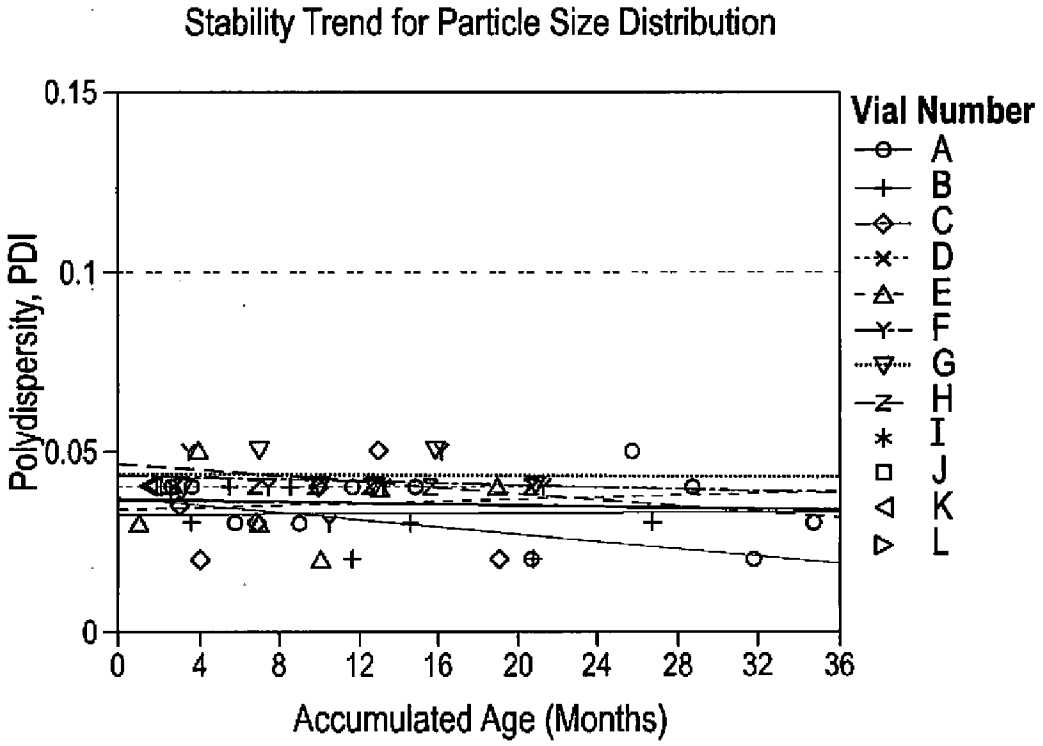

Stability Trend for Particle Size Distribution
Polydispersity, PDI
0.15
0.1
0.05
0
0
4
8
12
16
20
24
28
32
36
Accumulated Age (Months)
Vial Number
A
B
C
D
E
F
G
H
I
J
K
L

FIG. 10

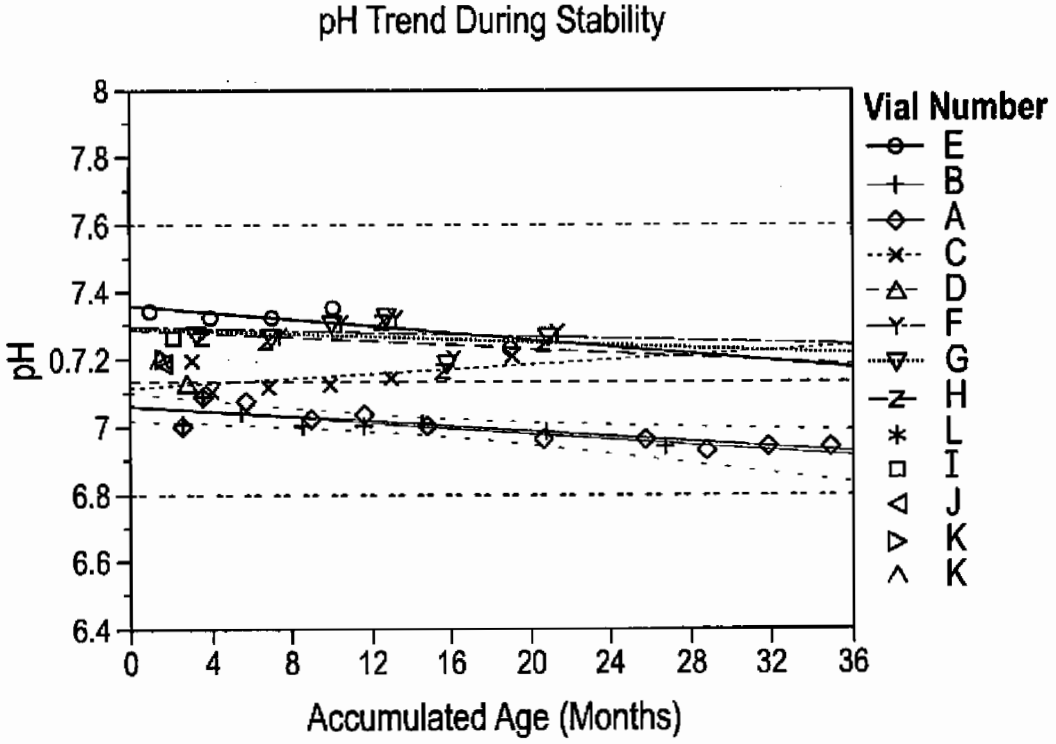

pH Trend During Stability
pH
8
7.8
7.6
7.4
7.2
7
6.8
6.6
6.4
0
4
8
12
16
20
24
28
32
36
Accumulated Age (Months)
Vial Number
E
B
A
C
D
F
G
H
L
I
J
K
K

FIG. 11A

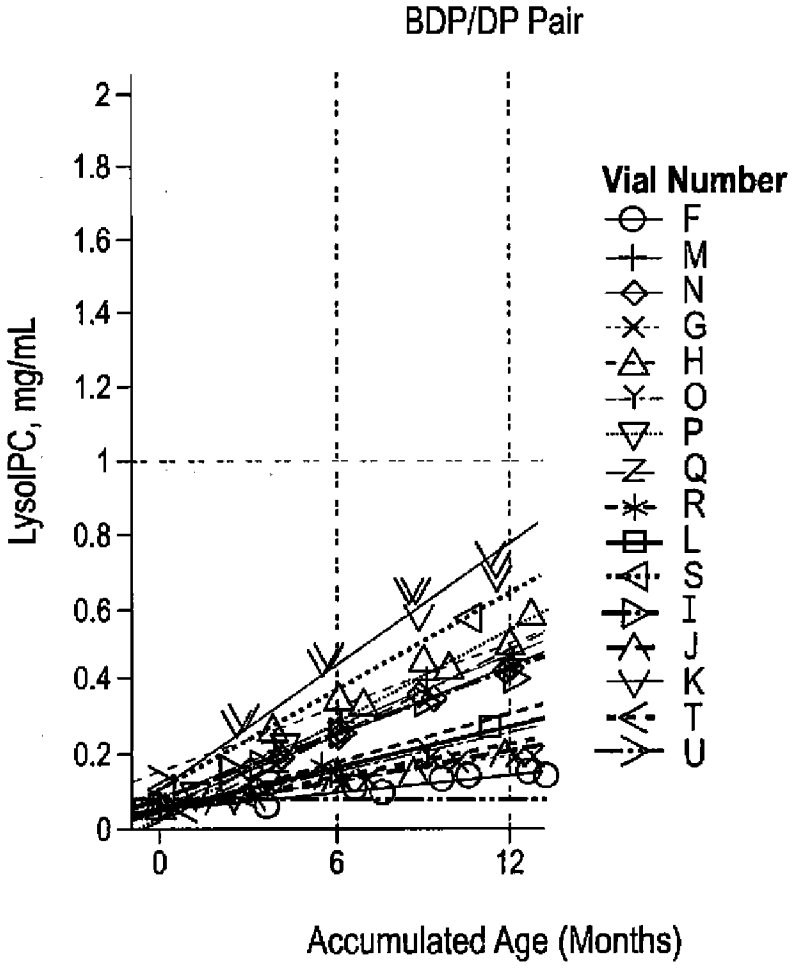

BDP/DP Pair
LysolPC, mg/mL
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
6
12
Accumulated Age (Months)
Vial Number
F
M
N
G
H
O
P
Q
R
L
S
I
J
K
T
U

FIG. 11B

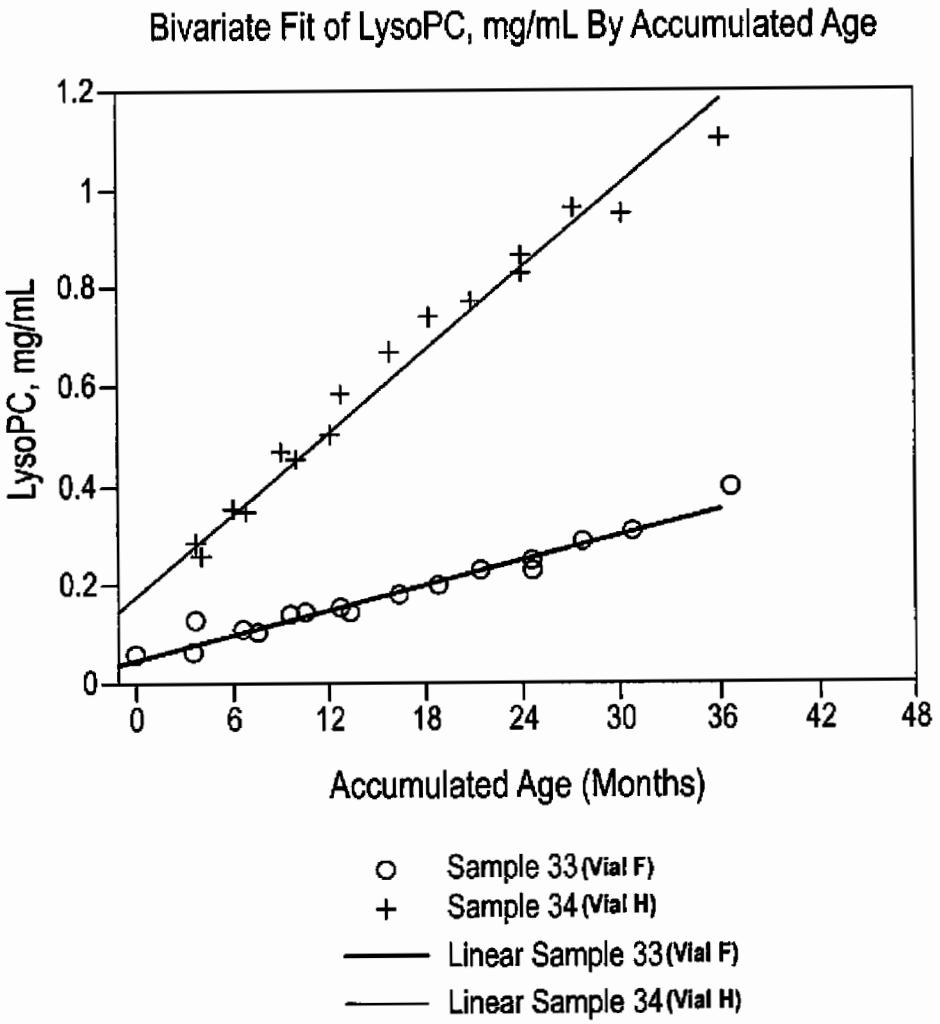

Bivariate Fit of LysoPC, mg/mL By Accumulated Age
LysoPC, mg/mL
1.2
1
0.8
0.6
0.4
0.2
0
0
6
12
18
24
30
36
42
48
Accumulated Age (Months)
Sample 33 (Vial F)
Sample 34 (Vial H)
Linear Sample 33 (Vial F)
Linear Sample 34 (Vial H)

FIG. 12

FIG. 13A

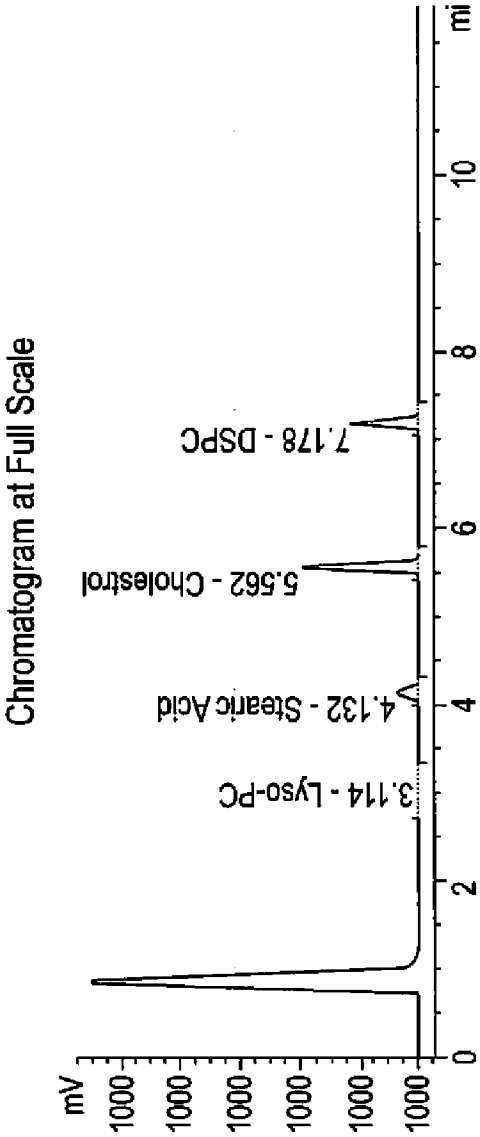
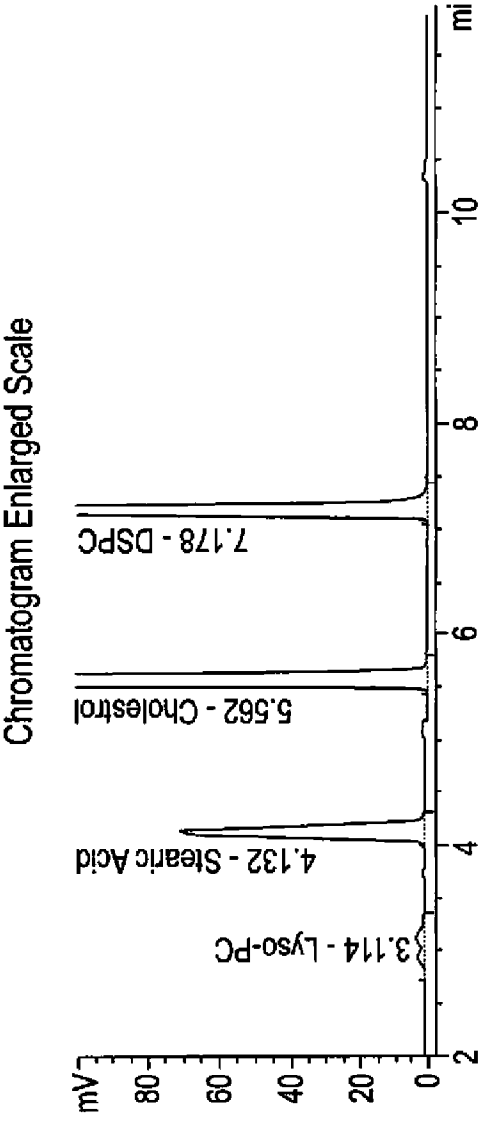


FIG. 13B



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/057247

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/127 A61K31/4745
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, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 8 658 203 B2 (DRUMMOND DARYL C [US] ET AL) 25 February 2014 (2014-02-25) cited in the application examples 11,13-15, 17, 82; table 11 column 22, lines 38-47 column 25, lines 9-21 column 26, lines 37-64 -----	1-23
Y	ZHONG Z ET AL: "Analysis of cationic liposomes by reversed-phase HPLC with evaporative light-scattering detection", JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS, NEW YORK, NY, US, vol. 51, no. 4, 11 March 2010 (2010-03-11), pages 947-951, XP026813988, ISSN: 0731-7085 [retrieved on 2009-10-09] page 951, left-hand column, paragraph 1 -----	1-23
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
15 December 2016	23/12/2016

Name and mailing address of the ISA/	Authorized officer
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AWA DICKO ET AL: "Intra and Inter-Molecular Interactions Dictate the Aggregation State of Irinotecan Co-Encapsulated with Floxuridine Inside Liposomes", PHARMACEUTICAL RESEARCH, KLUWER ACADEMIC PUBLISHERS-PLENUM PUBLISHERS, NL, vol. 25, no. 7, 5 March 2008 (2008-03-05), pages 1702-1713, XP019613128, ISSN: 1573-904X page 1703, left-hand column, paragraph 2 -----	1-23

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/057247

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 8658203 B2	25-02-2014	US 2007110798 A1	17-05-2007
		US 2014127136 A1	08-05-2014