View of NCT01523457 on 2012_01_31

ClinicalTrials Identifier: NCT01523457
Updated: 2012_01_31

Descriptive Information

Brief title Study of Modified FOLFIRINOX in Advanced Pancreatic Cancer

Official title Phase II Study of Modified FOLFIRINOX in Advanced Pancreatic Cancer

Brief summary

The primary objective of this study is to determine the progression free survival in patients with metastatic pancreatic cancer and in patients with locally advanced unresectable non-metastatic pancreatic cancer treated with a dose-attenuated modification of folinic acid, fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX). Secondary endpoints include: determine objective response rate according to RECIST; determine overall survival; evaluate toxicity; determine rate of resection in locally advanced unresectable stratum; correlate time to progression, objective response, and overall survival with early changes in glucose metabolism using [18F]-fluorodeoxyglucose (FDG)-positron emission tomography (PET) scanning.

Detailed description

Phase Phase 2
Study type Interventional
Study design Treatment
Study design Open Label
Study design Single Group Assignment
Study design Efficacy Study
Primary outcome Measure: Progression free survival
Time Frame: 24 weeks
Safety Issue? No
Description:

The primary objective of this study is to determine the progression free survival in patients with metastatic pancreatic cancer and in patients with locally advanced unresectable non-metastatic pancreatic cancer treated with a dose-attenuated modification of FOLFIRINOX.

Secondary outcome Measure: Objective response rate
Time Frame: 24 weeks

	Safety Issue? No Description: Response will be assessed by Response Evaluation Criteria in Solid Tumors (RECIST) at 8 week intervals in patients with metastatic disease and in patients with locally advanced disease.
Secondary outcome	Measure: Overall survival Time Frame: 24 weeks Safety Issue? No Description: Overall survival will be determined in patients with metastatic disease and in patients with locally advanced disease.
Secondary outcome	Measure: Toxicity Time Frame: 24 weeks Safety Issue? No Description: Toxicities will be assessed according to Common Terminology Criteria for Adverse Events (CTCAE) 4.0. Rates of grade 3 and 4 toxicities will be compared to historical controls (Conroy T, Desseigne F, Ychou M, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med 2011;364:1817-25.)
Secondary outcome	Measure: Rate of resection in patients with locally advanced unresectable disease Time Frame: 24 weeks Safety Issue? No Description: The rate of surgical resection in the cohort of patients with locally advanced unresectable disease will be determined.
Secondary outcome	Measure: Correlate time to progression, objective response, and overall survival with early changes in glucose metabolism using FDG-positron emission tomography (PET) scanning Time Frame: 24 weeks Safety Issue? No Description: The time to progression, objective response rate, and overall survival will be correlated with early changes in glucose metabolism using FDG-positron emission tomography (PET) scanning in patients with metastatic disease and locally advanced disease.
Enrollment	67 (Anticipated)
Condition	Metastatic Pancreatic Cancer

Condition	Pancreatic Cancer
Intervention	Drug: Folfirinox • Oxaliplatin 85 mg/m2 IV infused over two hours, followed by • Leucovorin 400 mg/m2 IV over two hours • Irinotecan 135 mg/m2 IV over 90 minutes (concurrent with leucovorin during the last 90 min of the leucovorin infusion) • 5-FU 300mg/m2 IV bolus, then 2400 mg/m2 continuous IV infusion over 46 hours

Recruitment Information

Status	Recruiting
Start date	2011-10
Last follow-up date	2014-06 (Anticipated)
Primary completion date	2013-12 (Anticipated)

Criteria

Inclusion Criteria:
- Pathologic or cytologic documentation of pancreatic adenocarcinoma
- Metastatic or locally advanced unresectable disease, including borderline unresectable disease
- Patients with biliary or gastroduodenal obstruction must have drainage or surgical bypass prior to starting chemoradiation
- Measurable or non-measurable assessable disease
- No prior treatment (chemotherapy, biological therapy, or radiotherapy) for metastatic or non-metastatic locally advanced unresectable pancreatic cancer
- 6 months since completion of any prior neoadjuvant or adjuvant therapy (chemotherapy or radiotherapy) for resected pancreatic cancer
- No prior treatment with oxaliplatin or irinotecan
- No prior treatment with fluoruouracil or capecitabine unless administered as a radiosensitizing drug during adjuvant/neoadjuvant chemoradiotherapy after/before resection of pancreatic cancer
- Patients who received chemotherapy > 2 years ago for malignancies other than pancreatic cancer are eligible, provided that chemotherapy was completed > 2 years ago and there is no evidence of the second malignancy at the time of study entry
- > 4 weeks since major surgery
- No other concurrent anticancer therapy
- ECOG Performance Status: 0-1
- Age > 18
- No other malignancy within past two years except basal cell carcinoma of the skin, cervical carcinoma in situ, or nonmetastatic prostate cancer
- Paraffin block or slides must be available
- Adequate organ function

- No interstitial pneumonia or extensive and symptomatic interstitial fibrosis of the lung
- No > grade 1 sensory peripheral neuropathy
- No uncontrolled seizure disorder, active neurological disease, or known CNS disease
- No significant cardiac disease, including the following: unstable angina, New York Heart Association class II-IV congestive heart failure, myocardial infarction within six months prior to study enrollment
- No history of chronic diarrhea
- Not pregnant and not nursing
- No other medical condition or reason that, in the opinion of the investigator, would preclude study participation
- Laboratory parameters as follows: absolute neutrophil count ≥ 1,500/uL, platelet count ≥ 100,000/uL, hemoglobin ≥ 9 g,/dL, creatinine < 1.5 X ULN or estimated GFR > 30 ml/min, bilirubin < 1.5 X ULN, AST and ALT < 3 X ULN, negative pregnancy test in women of childbearing age

Gender	Both
Minimum age	18 Years
Healthy volunteers	No

Administrative Data

Organization name	Yale University
Organization study ID	1108008901
Sponsor	Yale University
Health Authority	United States: Institutional Review Board

View of NCT01643499 on 2012_07_17

ClinicalTrials Identifier: NCT01643499
Updated: 2012_07_17

Descriptive Information

Brief title Genotype-guided Dosing of mFOLFIRINOX Chemotherapy in Patients With Previously Untreated Advanced Gastrointestinal Malignancies

Official title A Genotype-guided Dosing Study of mFOLFIRINOX in Previously Untreated Patients With Advanced Gastrointestinal Malignancies

Brief summary

This study is being done to determine the dose of a chemotherapy drug (irinotecan [irinotecan hydrochloride]) that can be tolerated as part of a combination of drugs. There is a combination of chemotherapy drugs often used to treat gastrointestinal cancer, which consists of 5-FU (fluorouracil), leucovorin (leucovorin calcium), irinotecan and oxaliplatin and is known as "FOLFIRINOX". FOLFIRINOX is a current drug therapy combination (or regimen) used for people with advanced pancreatic cancer, although this combination is not Food and Drug Administration (FDA) approved for this indication. FOLFIRINOX was recently shown in a separate clinical trial to increase survival compared to another commonly used drug in pancreatic cancer called gemcitabine. FOLFIRINOX is also a reasonable regimen for those with other advanced cancers of the gastrointestinal tract, including colon cancer, rectal cancer, esophagus cancer, stomach cancer, gall bladder cancer, bile duct cancer, ampullary cancer, and cancers with an unknown primary location. The best dose of irinotecan to use in FOLFIRINOX is not known. This study will analyze one gene (uridine 5'-diphospho [UDP] glucuronosyltransferase 1 family, polypeptide A1 [UGT1A1] gene) of subjects for the presence of an alteration in that gene, which may affect how the body handles irinotecan. Genes help determine some of the investigators individual characteristics, such as eye color, height and skin tone. Genes may also determine why people get certain diseases and how medicines may affect them. The result of the genetic analysis will divide subjects into one of three groups: A, B, or C. Group A (approximately 45% of subjects) will receive the standard dose of irinotecan. Group B (approximately 45% of subjects) will receive a lower dose of irinotecan. Group C (approximately 10% of subjects) will receive an even lower dose of irinotecan

Detailed description

PRIMARY OBJECTIVES:
I. To determine the dose-limiting toxicity (DLT) rate in cycle #1 in each of two UGT1A1 genotype groups (*1*1, *1*28) using genotype-guided dosing of

irinotecan as part of the modified (m) FOLFIRINOX regimen.

SECONDARY OBJECTIVES:
I. To determine the cumulative dose intensity of irinotecan achieved in each genotype group.
II. To determine the response rates by Response Evaluation Criteria in Solid Tumors (RECIST) (version 1.1) for each different disease (pancreatic cancer, biliary cancers, gastric cancer, colorectal cancer, adenocarcinoma of unknown primary) treated in the study.

OUTLINE:
Patients receive oxaliplatin intravenously (IV) over 2 hours, irinotecan hydrochloride IV over 1.5 hours, leucovorin calcium IV over 2 hours, and fluorouracil IV continuously over 46 hours on days 1 and 15. Treatment repeats every 4 weeks for up to 6 courses in the absence of disease progression or unacceptable toxicity.

Phase	Phase 1
Study type	Interventional
Study design	Treatment
Study design	Open Label
Study design	Single Group Assignment
Study design	Safety Study
Primary outcome	Measure: DLT rate in course 1 for each of the two most common genotype groups (*1*1 and *1*28) Time Frame: 4 weeks Safety Issue? Yes Description: To show that the DLT rate is less than 33% with at least 70-80% confidence, which is comparable to the standard 3+3 phase I design with 0 out of 3 or 1 out of 6 patients experiencing a DLT.
Secondary outcome	Measure: Response rates (by RECIST 1.1) for patients with each different type of gastrointestinal malignancy Time Frame: Up to 1 year Safety Issue? No
Secondary outcome	Measure: Cumulative dose intensity of irinotecan hydrochloride Time Frame: Up to 1 year Safety Issue? No
Enrollment	45 (Anticipated)
Condition	Acinar Cell Adenocarcinoma of the Pancreas
Condition	Adenocarcinoma of the Gallbladder
Condition	Adenocarcinoma of Unknown Primary
Condition	Adult Primary Cholangiocellular Carcinoma
Condition	Advanced Adult Primary Liver Cancer
Condition	Cholangiocarcinoma of the Extrahepatic Bile Duct

Condition	Cholangiocarcinoma of the Gallbladder
Condition	Diffuse Adenocarcinoma of the Stomach
Condition	Duct Cell Adenocarcinoma of the Pancreas
Condition	Intestinal Adenocarcinoma of the Stomach
Condition	Localized Unresectable Adult Primary Liver Cancer
Condition	Metastatic Carcinoma of Unknown Primary
Condition	Metastatic Extrahepatic Bile Duct Cancer
Condition	Mixed Adenocarcinoma of the Stomach
Condition	Mucinous Adenocarcinoma of the Colon
Condition	Mucinous Adenocarcinoma of the Rectum
Condition	Newly Diagnosed Carcinoma of Unknown Primary
Condition	Signet Ring Adenocarcinoma of the Colon
Condition	Signet Ring Adenocarcinoma of the Rectum
Condition	Stage III Pancreatic Cancer
Condition	Stage IIIA Colon Cancer
Condition	Stage IIIA Gallbladder Cancer
Condition	Stage IIIA Gastric Cancer
Condition	Stage IIIA Rectal Cancer
Condition	Stage IIIB Colon Cancer
Condition	Stage IIIB Gallbladder Cancer
Condition	Stage IIIB Gastric Cancer
Condition	Stage IIIB Rectal Cancer
Condition	Stage IIIC Colon Cancer
Condition	Stage IIIC Gastric Cancer
Condition	Stage IIIC Rectal Cancer
Condition	Stage IV Gastric Cancer
Condition	Stage IV Pancreatic Cancer
Condition	Stage IVA Colon Cancer
Condition	Stage IVA Gallbladder Cancer
Condition	Stage IVA Rectal Cancer
Condition	Stage IVB Colon Cancer
Condition	Stage IVB Gallbladder Cancer
Condition	Stage IVB Rectal Cancer
Condition	Unresectable Extrahepatic Bile Duct Cancer
Arm/Group	Arm Label: Treatment (mFOLFIRINOX) Experimental Patients receive oxaliplatin IV over 2 hours on, irinotecan hydrochloride IV over 1.5 hours, leucovorin calcium IV over 2 hours, and fluorouracil IV continuously over 46 hours on days 1 and 15. Treatment repeats every 4 weeks for up to 6 courses in the absence of disease progression or unacceptable toxicity.

Intervention	Drug: oxaliplatin (mFOLFIRINOX) Arm Label: Treatment Given IV
Intervention	Drug: irinotecan hydrochloride (mFOLFIRINOX) Arm Label: Treatment Given IV
Intervention	Drug: leucovorin calcium (mFOLFIRINOX) Arm Label: Treatment Given IV
Intervention	Drug: fluorouracil (mFOLFIRINOX) Arm Label: Treatment Given IV
Intervention	Other: laboratory biomarker analysis Arm Label: Treatment (mFOLFIRINOX) Correlative studies

Recruitment Information

Status	Recruiting
Start date	2012-03
Last follow-up date	2014-03 (Anticipated)
Primary completion date	2013-03 (Anticipated)

Criteria

Inclusion Criteria:
- Histologically or cytologically confirmed locally advanced or metastatic pancreatic adenocarcinoma, colorectal adenocarcinoma, gastric adenocarcinoma, cholangiocarcinoma, gall bladder adenocarcinoma, ampullary carcinoma, adenocarcinoma of unclear primary (with a gastrointestinal primary suspected), or other primary gastrointestinal malignancy for which the treating physician feels that mFOLFIRINOX is a reasonable therapeutic option.
- Patients with a history of obstructive jaundice due to the primary tumor must have a metal biliary stent in place,
- Eastern Cooperative Oncology Group (ECOG) performance status =< 1,
- Life expectancy > 3 months,
- Absolute neutrophil count (ANC) >= l500/ul,
- Hemoglobin >= 9g/dL,
- Platelets >= 100,000/ ul,
- Total bilirubin < 1.5 x upper limit of normal,
- Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) < 2.5 x upper limit of normal for patients without liver metastases OR SGOT and SGPT < 5 x upper limit of normal for patients with liver metastases,
- Creatinine =< 1.5 x upper limit of normal,
- Measurable or non-measurable disease will be allowed,

- Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation, up until 30 days after final study treatment; should a woman become pregnant or suspect that she is pregnant while participating in this study, she should inform her treating physician immediately,
- Patients taking substrates, inhibitors, or inducers of Cytochrome P450 3A4 (CYP3A4) should be encouraged to switch to alternative drugs whenever possible, given the potential for drug-drug interactions with irinotecan
- Signed informed consent.

Exclusion Criteria:
- Prior chemotherapy or radiation therapy for any cancer,
- Inflammatory bowel disease (Crohn's disease, ulcerative colitis),
- Diarrhea, grade 1 or greater by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE, v. 4.0); pancreatic cancer patients with clinical evidence of pancreatic insufficiency must be taking pancreatic enzyme replacement,
- Neuropathy, grade 2 or greater by NCI-CTCAE, v. 4.0,
- Documented brain metastases,
- Serious underlying medical or psychiatric illnesses that would, in the opinion of the treating physician, substantially increase the risk for complications related to treatment,
- Active uncontrolled bleeding,
- Pregnancy or breastfeeding,
- Major surgery within 4 weeks,
- Previous or concurrent malignancy, except for adequately treated basal cell or squamous cell skin cancer, in situ cervical cancer, or any other cancer for which the patient has been previously treated and the lifetime recurrence risk is less than 30%,
- Patients with any polymorphism in UGT1A1 other than *1 or *28.

Gender	Both
Minimum age	18 Years
Healthy volunteers	No

Administrative Data

Organization name	University of Chicago
Organization study ID	12-0033
Secondary ID	NCI-2012-00585 (CTRP (Clinical Trial Reporting Program))
Sponsor	University of Chicago
Collaborator	National Cancer Institute (NCI)
Health Authority	United States: Institutional Review Board

View of NCT01688336 on 2012_09_18

ClinicalTrials Identifier: NCT01688336
Updated: 2012_09_18

Descriptive Information

Brief title FOLFIRINOX for Unresectable Locally Advanced and Borderline Resectable Pancreatic Cancer

Official title Phase II Single Arm Clinical Trial of FOLFIRINOX for Unresectable Locally Advanced and Borderline Resectable Pancreatic Cancer

Brief summary

This single arm, multi-center phase II clinical trial will assess the safety and efficacy of FOLFIRINOX in the first-line setting in patients with unresectable locally advanced (ULA) and borderline resectable (BR) pancreatic cancer.

Detailed description

FOLFIRINOX regimen was recently presented at an international oncology meeting and represents a new standard in the treatment of metastatic pancreatic cancer for selected patients. With improved overall survival (OS) and response rates (RR) in the metastatic setting, we hypothesize that in patients with less tumor burden, this regimen will be safe and well tolerated, improve OS, progression free survival (PFS), and RR, and improve resectability rates, as compared to historical data from standard single agent gemcitabine therapy for unresectable locally advanced (ULA) patients and standard radiation with concurrent 5 flourouracil (5FU) chemotherapy for borderline resectable (BR) patients. While both ULA and BR patients will be eligible for the present study, our primary objective concerns ULA patients, and we plan to enroll 45 patients in this group.

Patients meeting eligibility criteria will be consented and treated with FOLFIRINOX every 2 weeks (1 cycle = 4 weeks = 2 treatments). Patients will undergo repeat imaging (CT or MRI) every 2 cycles and reassessed for resectability of the tumor. All patients that are not able to undergo surgical resection, due to insufficient down-staging or patient preference, will continue on protocol-based therapy until disease progression, unacceptable toxicity, study withdrawal, or death.

Phase Phase 2
Study type Interventional
Study design Treatment
Study design Open Label
Study design Single Group Assignment

Study design	Efficacy Study
Primary outcome	Measure: Median overall survival (OS) of FOLFIRINOX in patients with unresectable locally advanced (ULA) pancreatic cancer Time Frame: Up to 3 years Safety Issue? No Description: All patients who receive at least Day 1 of FOLFIRINOX treatment will be evaluable and followed up for up to 3 years for the primary outcome of overall survival (OS).
Secondary outcome	Measure: Overall survival for borderline resectable patients Time Frame: Up to 3 years Safety Issue? No Description: All patients who receive at least Day 1 of FOLFIRINOX treatment will be evaluable and followed up for up to 3 years for the outcome of overall survival (OS)
Secondary outcome	Measure: Progression free survival (PFS) Time Frame: D1 of treatment until evidence of tumor progression Safety Issue? No Description: Progression free survival will be measured from D1 of treatment until evidence of tumor progression (including clinical deterioration related to the underlying pancreatic cancer, as assessed by the investigator) or death from any cause. Patients that are lost to follow-up will be censored
Secondary outcome	Measure: Objective response rate Time Frame: Up to 3 years Safety Issue? No Description: All patients who have received at least one cycle of treatment and have their disease reevaluated will be evaluable for assessment of objective response and will be followed for up to 3 years for survival. Disease will be evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST, version 1.1) every eight weeks and at the time of disease progression by the same modality as the baseline evaluation: CT abdomen and chest (preferable) or MRI abdomen and CT chest. Objective response rate will be measured by the number of complete responses (CR) and partial responses (PR), as determined by RECIST 1.1 criteria.

	Patients who drop out of the study prior to disease evaluation will not be evaluable for response unless the patient undergoes radiologic evaluation or their disease progresses clinically.
Secondary outcome	Measure: Disease control rate (DCR) Time Frame: Up to 3 years Safety Issue? No Description: Disease control rate will be measured by the rate of rate of radiographic complete responses (CR) and partial responses (PR) and stable disease (SD), as determined by RECIST 1.1 criteria.
Secondary outcome	Measure: Rate of resectability (RR) Time Frame: Up to 3 years Safety Issue? No Description: Rate of resectability will be evaluated by determining the number of patients who were initially deemed to have ULA or BR disease and, following any period of treatment, were subsequently deemed to have resectable disease and undergo surgical resection. The denominator will reflect all patients with ULA or BR disease.
Enrollment	45 (Anticipated)
Condition	Pancreatic Cancer
Arm/Group	Arm Label: FOLFIRINOX Experimental FOLFIRINOX given to all subjects
Intervention	Drug: FOLFIRINOX Arm Label: FOLFIRINOX FOLFIRINOX will be given intravenously on Days 1, 15, and 28 of each 28 day cycle. Drugs are given in combination in this order: -Oxaliplatin (85 mg/m2) -Leucovorin (400mg/ m2) -Irinotecan (180 mg/m2) -5FU (400mg/m2)bolus then 2400 mg/m2 over 46 hours
URL	http://unclineberger.org/
URL	http://www.cancer.gov/
See also	UNC Lineberger Comprehensive Cancer Center homepage
See also	National Cancer Institute (NCI) homepage

Recruitment Information

Status	Recruiting
Start date	2012-01

Last follow-up date 2016-02 (Anticipated)
Primary completion date 2015-01 (Anticipated)
Criteria

Inclusion Criteria:

-Biopsy confirmed adenocarcinoma of the pancreas.
-Measurable or non-measurable but evaluable (as determined by Response Evaluation Criteria in Solid Tumors version 1.1 [RECIST 1.1]) unresectable locally advanced (ULA) or borderline resectable (BR) disease that is not amenable to curative intent therapy. Baseline CT abdomen and chest (or MRI abdomen) within 28 days prior to initiation of FOLFIRINOX is required.
-ECOG performance status 0 or 1.
-No prior chemotherapy or chemoradiotherapy for pancreatic cancer.
-Age ≥ 18 years of age.
-Laboratory requirements at study entry:
--Hemoglobin ≥ 10 g/dL (transfusions are acceptable)
--ANC ≥ 1.5 x 109/L
--Platelets ≥ 100 x 109/L
--Creatinine ≤ 1.5 x ULN, or creatinine clearance ≥ 50 mL/min (estimated by Cockcroft-Gault or measured)
--Total bilirubin ≤ 1.5 x ULN
--AST/ALT ≤ 3 x ULN
--GGT ≤ 5 x ULN
-Life expectancy of at least 6 months.
-Women of childbearing potential (WOCBP) must have a negative serum or urine pregnancy test performed within 14 days prior to initiation of FOLFIRINOX.
-WOCBP and men must agree to use adequate contraception prior to study entry, for the duration of study participation, and 8 weeks after the end of treatment.
-Before patient registration, written informed consent must be given.

Exclusion Criteria:
-Local recurrence or resectable recurrence of pancreatic cancer.
-Other malignancies within the past 3 years except for adequately treated cervical or vulvar carcinoma in situ, treated basal cell carcinoma, superficial bladder tumors (Ta, Tis & T1). Any cancer curatively treated >3 years prior to entry is permitted.
-Hypersensitivity to 5FU, oxaliplatin or other platinum agent, or irinotecan or to their excipients. Known dihydropyrimidine dehydrogenase (DPD) enzyme deficiency.
-Participation in any investigational drug study within 4 weeks preceding the start of study treatment. Patients are not permitted to participate in another investigational drug study while being treated on this protocol.
-Cardiac disease: Congestive heart failure symptoms > class II NYHA. Unstable angina (anginal symptoms at rest) or new onset angina beginning within the last 3 months. Myocardial infarction within the past 6 months. Cardiac ventricular arrhythmias requiring anti-arrhythmic therapy.
-History of or suspected Gilbert's Disease (baseline testing not required).
-Baseline peripheral neuropathy/paresthesia grade ≥ 1.

-Active hepatitis B, unless patient has been on stable meds for at least 2 months (baseline testing not required).
-Active clinically serious infections (> grade 2).
-Any other hemorrhage/bleeding event > CTCAE Grade 3 within the 12 weeks prior to the first dose FOLFIRINOX.
-Evidence or history of bleeding diathesis or coagulopathy. NOTE: If therapeutic anticoagulation required, the investigator is encouraged to switch patient to (or maintain on) low molecular weight heparin during the trial.
-Major surgery, open biopsy or significant traumatic injury within 8 weeks of first study drug. A core pancreatic or liver biopsy does not preclude the patient from the study.
-Unable or unwilling to discontinue use of ketoconazole or St John's wort. Use of CYP3A4 enzyme-inducing drugs and strong CYP3A4 inhibitors is discouraged, but not contraindicated.
-Active drug or alcohol abuse.
-Pregnant or lactating women.
-Psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule; those conditions should be discussed with the patient before registration in the trial.

Gender	Both
Minimum age	18 Years
Healthy volunteers	No

Administrative Data

Organization name	UNC Lineberger Comprehensive Cancer Center
Organization study ID	LCCC 1105
Sponsor	UNC Lineberger Comprehensive Cancer Center
Health Authority	United States: Institutional Review Board

View of NCT01771146 on 2013_01_17

ClinicalTrials Identifier: NCT01771146
Updated: 2013_01_17

Descriptive Information

Brief title Neoadjuvant FOLFIRINOX Regimen in Patients With Non-metastatic Pancrease Cancer

Official title A Prospective Evaluation of Neoadjuvant FOLFIRINOX Regimen in Patients With Non-metastatic Pancreas Cancer (Baylor University Medical Center and Texas Oncology Experience)

Brief summary

A prospective evaluation of neoadjuvant FOLFIRINOX regimen in patients with non-metastatic pancreas cancer (Baylor University Medical Center and Texas Oncology Experience)

Detailed description

Phase N/A

Study type Interventional

Study design Treatment

Study design Open Label

Study design Single Group Assignment

Primary outcome Measure: Progression Free Survival (PFS) as defined by the length of time that a patient survives without any signs or symptoms of that cancer or any other type of cancer
Time Frame: Up to 5 years
Safety Issue? No

Secondary outcome Measure: • The length of time from diagnosis (enrollment) to death
Time Frame: Up to 5 years
Safety Issue? No

Secondary outcome Measure: • Overall Survival rate defined by the % of people who are alive for a certain period of time after diagnosis
Time Frame: Up to 5 years
Safety Issue? No

Secondary outcome Measure: • R0 resection as defined as microscopically negative margins
Time Frame: Up to 5 years
Safety Issue? No

Enrollment 100 (Anticipated)

Condition	Localized Pancreas Cancer
Condition	Non-metastatic Pancreas Cancer
Arm/Group	Arm Label: Neoadjuvant FOLFIRINOX Regimen Other
Intervention	Other: FOLFIRINOX Regimen: Eloxatin (Oxaliplatin) Camptosar (Irinotecan Hydrochloride) Adrucil (Fluorouracil; 5-FU) Arm Label: Neoadjuvant FOLFIRINOX Regimen Eloxatin® (Oxaliplatin) 85 mg per square meter 2-hour IV infusion Camptosar® (Irinotecan Hydrochloride) 180 mg per square meter 90-minute IV infusion via Y-connector adrucil (Fluorouracil; 5-FU)2400 mg per square meter 46-hour IV infusion

Recruitment Information

Status	Recruiting
Start date	2012-10
Last follow-up date	2017-09 (Anticipated)
Primary completion date	2017-09 (Anticipated)

Criteria

Inclusion Criteria:
- 18 years of age or older
- Male or non-pregnant and non-lactating female
- Histologically or cytologically confirmed adenocarcinoma of pancreas
- Patients must have satisfactory blood counts and blood chemistry levels at baseline (refer to Appendix 2, Study Laboratory References Range).
- Patient has Eastern Cooperative Oncology Group(ECOG) Performance Status 0 to 1 (refer to Appendix 7):
- 0 - Asymptomatic (Fully active, able to carry on all predisease activities without restriction)
- 1 - Symptomatic but completely ambulatory (Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature. For example, light housework, office work)
- Signed study consent form

Exclusion Criteria:
- <18 years of age
- Pregnant or lactating female
- Patient has islet cell neoplasms
- Patient has known brain metastases
- Patient has metastatic disease
- Active secondary malignancies
- Active, uncontrolled bacterial, viral, or fungal infection(s) requiring systemic therapy
- Known infection with hepatitis B, hepatitis C, or cirrhosis

- Major surgery or vascular device placement (excluding ports for IV medication/chemotherapy) within 2 weeks prior to Day 1 of treatment in study
- Prior chemotherapy or radiation for pancreatic cancer
- History of allergy or hypersensitivity to the study drugs
- Patient is enrolled in any outside (outside Baylor University Medical Center or Texas Oncology) clinical protocol or investigational trial
- Significant cardiac disease as defined as New York Heart Association (NYHA) classification III or IV, uncontrolled congestive heart failure (CHF), or prior myocardial infarction (MI) last 6-months
- Any prior gastrointestinal (GI) disease or history of prior pelvic or abdominal radiation which in the opinion of the investigator may place the patient at increased risk
- Peripheral sensory neuropathy ≥ to grade 2 at baseline
- Significant co-morbidities deemed by investigator as unsuitable for participation/enrollment
- Study consent form not signed

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Gender	Both
Minimum age	18 Years
Healthy volunteers	No

Administrative Data

Organization name	Baylor Research Institute
Organization study ID	012-180
Sponsor	Baylor Research Institute
Health Authority	United States: Institutional Review Board

View of NCT01926197 on 2013_08_19

ClinicalTrials Identifier: NCT01926197
Updated: 2013_08_19

Descriptive Information

Brief title
Phase III FOLFIRINOX (mFFX) +/- SBRT in Locally Advanced Pancreatic Cancer

Official title
A Randomized Phase III Study Evaluating Modified FOLFIRINOX (mFFX) With or Without Stereotactic Body Radiotherapy (SBRT) in the Treatment of Locally Advanced Pancreatic Cancer

Brief summary

A Pancreatic Cancer Radiotherapy Study Group (PanCRS) Trial

Primary Objective:
To determine progression free survival for mFFX +/- SBRT.

Secondary Objectives:
* To determine metastasis free survival following mFFX chemotherapy alone or with SBRT.
* To determine the overall survival in pancreatic cancer patients treated with chemotherapy +/- SBRT.
* To determine local progression-free survival in pancreatic cancer patients after chemotherapy +/- SBRT.
* To evaluate acute (within 3 months of treatment) grade 2 or greater gastritis, fistula, enteritis, or ulcer and any other grade 3-4 gastrointestinal toxicity within 3 months of treatment.
* To evaluate the utility of FDG-PET for treatment planning and estimation of progression free survival.
* To identify new biomarkers in pancreatic cancer.
* To evaluate the quality of life of patients before and after either chemotherapy or chemotherapy and SBRT.

Detailed description

A Pancreatic Cancer Radiotherapy Study Group (PanCRS) Trial

Primary Objective:
To determine progression free survival for mFFX +/- SBRT.

Secondary Objectives:
* To determine metastasis free survival following mFFX chemotherapy alone or with SBRT.

* To determine the overall survival in pancreatic cancer patients treated with chemotherapy +/- SBRT.
* To determine local progression-free survival in pancreatic cancer patients after chemotherapy +/- SBRT.
* To evaluate acute (within 3 months of treatment) grade 2 or greater gastritis, fistula, enteritis, or ulcer and any other grade 3-4 gastrointestinal toxicity within 3 months of treatment.
* To evaluate the utility of FDG-PET for treatment planning and estimation of progression free survival.
* To identify new biomarkers in pancreatic cancer.
* To evaluate the quality of life of patients before and after either chemotherapy or chemotherapy and SBRT.

Phase	Phase 3
Study type	Interventional
Study design	Treatment
Study design	Parallel Assignment
Primary outcome	Measure: difference in progression-free survival between mFOLFIRINOX alone vs. mFOLFIRINOX and SBRT Time Frame: one year Safety Issue? No
Enrollment	172 (Anticipated)
Condition	Pancreatic Cancer
Arm/Group	Arm Label: mFFX Active Comparator mFOLFIRINOX
Arm/Group	Arm Label: mFFX+SBRT Experimental mFOLFIRINOX + SBRT
Intervention	Drug: Oxaliplatin Arm Label: mFFX
Intervention	Radiation: SBRT Arm Label: mFFX+SBRT
Intervention	Drug: Irinotecan Arm Label: mFFX
Intervention	Drug: Leucovorin Arm Label: mFFX
Intervention	Drug: 5FU Arm Label: mFFX

Recruitment Information

Status	Recruiting
Start date	2013-08
Primary completion date	2018-09 (Anticipated)
Criteria	

Inclusion Criteria:
• Histologically confirmed adenocarcinoma of the pancreas.
• Induction mFolfirinox no more than 2 cycles.
• Stable or better disease on re-staging scans following induction mFolfirinox. Determined unresectable by a pancreatic cancer surgeon or a multi-disciplinary or gastrointestinal oncology Tumor Board.
• SBRT treatment plan must satisfy all normal tissue constraints. Minor protocol deviations will be considered on an individual basis and must be approved by Coordinating Center Principal Investigator or Co-Investigator.
• Age >18 years.
• Karnofsky >70% (see Appendix II).
• Acceptable organ and marrow function (as defined in Section 3.1).
• Women who are not post-menopausal (defined in Appendix III) should have a negative urine or serum pregnancy test.
• Women and men of childbearing potential must agree to use adequate contraception for the duration of study participation.

Exclusion Criteria:
• No metastatic disease.
• No prior upper abdominal or liver radiation therapy.
• Chemotherapy other than 2 cycles mFolfirinox.
• No uncontrolled illness or active infection requiring systemic antibiotic treatment.
No concurrent malignancies other than non-melanoma skin cancer, non-invasive bladder cancer and carcinoma in situ of the cervix; and no other cancers within 5 years.

Gender	Both
Minimum age	18 Years
Healthy volunteers	No

Administrative Data

Organization name	Stanford University
Organization study ID	PANC0015
Secondary ID	27492
Sponsor	Stanford University
Health Authority	United States: Institutional Review Board

View of NCT01992705 on 2013_11_22

ClinicalTrials Identifier: NCT01992705
Updated: 2013_11_22

Descriptive Information

Brief title Borderline Pancreas Study: FOLFIRINOX +SBRT

Official title Neoadjuvant FOLFIRINOX and Stereotactic Body Radiotherapy (SBRT) Followed by Definitive Surgery for Patients With Borderline Resectable Pancreatic Adenocarcinoma: A Single-Arm Pilot Study

Brief summary

Primary Objective: To determine the rate of downstaging to resectability in patients with borderline resectable pancreatic cancer receiving FOLFIRINOX and SBRT as preoperative therapy.

Secondary Objective(s):
1)To assess the disease-free-survival, overall survival, time to recurrence and site of recurrence in patients with borderline resectable pancreatic cancer receiving preoperative FOLFIRINOX followed by SBRT
2)To investigate the safety and tolerability of FOLFIRINOX and SBRT in patients with resectable pancreatic cancer
3)To determine the radiologic and pathological response associated with preoperative SBRT and FOLFIRINOX therapy
4)To assess quality of life through and after treatment using the FACT-Hep questionnaire

Detailed description

The study investigators hypothesize that neoadjuvant FOLFIRINOX can be safely and efficaciously delivered using a sequential regimen with SBRT as an alternative to standard neoadjuvant chemoradiotherapy. Standard of care neoadjuvant treatment typically requires about six weeks of treatment with sub-systemic dosing of chemotherapy. The feasibility of the sequential delivery of the FOLFIRINOX followed by SBRT will be evaluated by capturing the prevalence of grade 3 toxicity and the treatment delay rate.

In our study, SBRT is planned sequentially to follow cycle 4 of chemotherapy treatment, provided toxicity has resolved to grade 2 or less. Thus, allowing for resolution of chemotherapy toxicity prior to initiation of radiation therapy. This interval and the fact that there is no concurrent delivery of chemo-RT, based on previously discussed experiences, including approaches where SBRT safely follows other intense chemotherapy regimens (see Polistina et al and Chuong [35,36]) makes this study feasible without establishing toxicity profile.

The proposed regimen of 4 cycles of FOLFIRINOX followed by 30 Gy/5 fractions using SBRT will be safely tolerated and will improve resectability rates in borderline resectable PDAC patients. In addition, this regimen will not compromise the ability to achieve a successful Whipple resection.

This regimen will improve the local control rate and overall disease free survival in this patient population. The investigators further hypothesize that early administration of FOLFIRNOX will provide optimal systemic therapy to control clinically occult micrometastases.

Phase	Phase 0
Study type	Interventional
Study design	Treatment
Study design	Open Label
Study design	Single Group Assignment
Study design	Safety/Efficacy Study
Primary outcome	Measure: Rate of downstaging to resectability in patients with borderline resectable pancreatic cancer receiving FOLFIRINOX and SBRT as preoperative therapy. Time Frame: Participants will be followed from randomization up to 120 months or death (from any cause) whichever comes first. Safety Issue? No Description: To determine the rate of downstaging to resectability in patients with borderline resectable pancreatic cancer receiving FOLFIRINOX and SBRT as preoperative therapy (AGGC 6th edition).
Secondary outcome	Measure: Survival status (disease-free-survival vs. overall survival) time to recurrence and site of recurrence in patients with borderline resectable pancreatic cancer receiving preoperative FOLFIRINOX followed by SBRT Time Frame: Participants will be followed from randomization up to 120 months or death (from any cause) whichever comes first. Safety Issue? No Description: To assess the disease-free-survival, overall survival, time to recurrence and site of recurrence in patients with borderline resectable pancreatic cancer receiving preoperative FOLFIRINOX followed by SBRT (RECIST)
Enrollment	20 (Anticipated)
Condition	Resectable Pancreatic Cancer
Arm/Group	Arm Label: Chemotherapy+SBRT prior to surgery if applicable Other

	FOLFIRINOX Drugs: -Calcium Folinate (Folinic Acid) 400 mg IV on Day 1 of each cycle (21d/cycle for a total of 4 cycles. Stereotactic Body Radiotherapy (SBRT): 30 Gy in 5 fractions given to radiographically defined pancreatic mass alone
Intervention	Other: Chemotherapy(FOLFIRINOX) + SBRT prior to surgery if applicable Arm Label: Chemotherapy+SBRT prior to surgery if applicable Patients will receive chemotherapy (21d/cycle for a total of 4 cycles) plus SBRT before screening for surgical resection of the pancreas.
Intervention	Drug: -Oxaliplatin 85 mg/m2 IV on Day 1 Arm Label: Chemotherapy+SBRT prior to surgery if applicable Oxaliplatin 85 mg/m2 IV on Day 1 of each cycle (21d/cycle for a total of 4 cycles).
Intervention	Drug: -Irinotecan 180 mg/m2 IV on Day 1 Arm Label: Chemotherapy+SBRT prior to surgery if applicable Irinotecan 180 mg/m2 IV on Day 1 of each cycle (21d/cycle for a total of 4 cycles).
Intervention	Drug: -5-FU (Fluorouracil) 2,400 mg/m2 IV over 46-48 hours Arm Label: Chemotherapy+SBRT prior to surgery if applicable 5-FU (Fluorouracil) 2,400 mg/m2 IV over 46-48 hours of each cycle (21d/cycle for a total of 4 cycles.

Recruitment Information

Status	Not yet recruiting
Start date	2014-01
Last follow-up date	2020-12 (Anticipated)
Primary completion date	2015-12 (Anticipated)

Criteria

Inclusion Criteria:
- ≥ 18 years at diagnosis.
- Biopsy proven pancreatic adenocarcinoma.
- Borderline resectable per NCCN criteria (No distant metastases, venous involvement of the portal vein/SMV, demonstrating tumor abutment and narrowing of the lumen, encasement of the portal vein/SMV without encasement of the nearby arteries, or short-segment venous occlusion resulting from either tumor thrombus or encasement but with suitable vessel proximal or distal to this area of vessel involvement, allowing for safe resection and reconstruction; gastroduodenal artery encasement up to the hepatic artery with either short segment encasement or direct abutment of the hepatic artery, without extension

to the celiac axis; tumor abutment of the SMA not to exceed 180 degrees of the circumference of the vessel wall.).
- Radiologically measurable or clinically evaluable disease.
- Pancreas protocol CT and/or MRI if required for further clarification of disease tissue planes within 4 weeks of registration.
- ECOG PS of 0-2.
- Able to get a Whipple resection per surgeon assessment performed within 4 weeks of registration.
- The following laboratory values obtained ≤ 28 days prior to registration:
  - Absolute neutrophil count (ANC) ≥ 1,500/mm3.
  - Platelet count ≥ 100,000/mm3.
  - Hemoglobin > 8.0 g/dL.
  - Total bilirubin ≤ 1.5 x upper limit of normal (ULN).
  - SGOT (AST) ≤ 2 x ULN.
  - SGPT (ALT) ≤ 2 x ULN.
  - Creatinine ≤ 1.5 x ULN.
  - CA 19-9 level (to establish baseline).
- A negative pregnancy test within 7 days prior to registration for women of childbearing potential. In addition, male and female participants must commit to adequate contraception while on study.
- Able to provide written informed consent.
- Willing to return for all required study assessments.
- Neurological assessment for pre-existing peripheral neuropathy.
- Documentation of pre-existing hearing deficits.

Exclusion Criteria:
- Any pancreatic adenocarcinoma that does not meet criteria for borderline resectable disease.
- Prior history of abdominal radiation therapy.
- History of autoimmune disease such as scleroderma, lupus, and inflammatory bowel disease.
- Patients with tumor-caused symptomatic bowel obstruction.
- Chemotherapy (including hormonal therapy) within the past 5 years from date of registration.
- Other invasive malignancies within the past 5 years from date of registration.
- Pregnant or nursing women or women of childbearing age that are unwilling to employ adequate contraception.
- Other co-morbid conditions which, based on the judgment of the physicians obtaining informed consent, would make the patient inappropriate for this study.

Gender	Both
Minimum age	18 Years
Healthy volunteers	No

Administrative Data

Organization name	University of Maryland
Organization study ID	HP-00055716
Sponsor	University of Maryland

Health Authority	United States: Institutional Review Board

View of NCT02028806 on 2014_01_06

ClinicalTrials Identifier: NCT02028806
Updated: 2014_01_06

Descriptive Information

Brief title mFOLFIRINOX as First-Line Chemotherapy in Treating Chinese Patients With Metastatic Pancreatic Cancer

Official title Phase II Trial to Investigate the Efficacy and Safety of mFOLFIRINOX in Patients With Metastatic Pancreatic Cancer in China

Brief summary

This phase II study was designed to evaluate the efficacy and safety of mFOLFIRINOX as first-line treatment for metastatic pancreatic cancer in China.

Detailed description

Although FOLFIRINOX regimen was recently presented to be effective for metastatic pancreatic cancer in selected patients who have good physical condition, there is still insufficient evidence on this regimen in treating patients with metastatic pancreatic cancer in China. Since for many tumors, different races may show different responses to the same regimen, we design this open, multicenter phase II study to evaluate the the efficacy and safety of mFOLFIRINOX as first-line treatment for metastatic pancreatic cancer in China.

Phase Phase 2

Study type Interventional

Study design Treatment

Study design Open Label

Study design Single Group Assignment

Study design Safety/Efficacy Study

Primary outcome Measure: Disease control rate
Time Frame: Up to 24 weeks
Safety Issue? No

Secondary outcome Measure: Progression free survival
Time Frame: From the date of first drug administration until the date of first documented progression or date of death from any cause, whichever came first, assessed up to 24 months
Safety Issue? No

Secondary outcome Measure: Overall survival
Time Frame: From the date of first drug administration until

	the date of death, assessed up to 60 months Safety Issue? No
Secondary outcome	Measure: Number of participants with AEs and SAEs as a measure of Safety Time Frame: Each follow up visit, assessed up to 24 weeks Safety Issue? Yes Description: Safety data will be assessed at each study visit using NCI CTCAE version 3.0
Secondary outcome	Measure: Quality of life Time Frame: Each follow up visit, assessed up to 24 weeks Safety Issue? No Description: Quality of life will be assessed at each study using EORTC QLQ-C30
Enrollment	40 (Anticipated)
Condition	Metastatic Pancreatic Cancer
Arm/Group	Arm Label: FOLFIRINOX Experimental Patients will receive mFOLFIRINOX every 2 weeks: Oxaliplatin 65 mg/m2 IV over 3 hours on Day 1; Irinotecan 150 mg/m2 IV over 90 minutes on Day 1; Leucovorin(l-LV) 200 mg/m2 IV over 2 hours on Day 1; followed by 5-Fluorouracil 2.4 g/m2 for 46 hours continuous infusion.
Intervention	Drug: mFOLFIRINOX Arm Label: FOLFIRINOX Patients will receive mFOLFIRINOX every 2 weeks: Oxaliplatin 65 mg/m2 IV over 3 hours on Day 1; Irinotecan 150 mg/m2 IV over 90 minutes on Day 1; Leucovorin(l-LV) 200 mg/m2 IV over 2 hours on Day 1; followed by 5-Fluorouracil 2.4 g/m2 for 46 hours continuous infusion.

Recruitment Information

Status	Recruiting
Start date	2013-02
Last follow-up date	2020-12 (Anticipated)
Primary completion date	2020-12 (Anticipated)

Criteria

Inclusion Criteria:

-Patients have provided a signed Informed Consent Form
- ECOG performance status of 0-1
- BMI ≥ 18.5
- Age: 18-65 years old

- Histologically confirmed diagnosis of metastatic pancreatic cancer
- No prior palliative chemotherapy
- Measurable disease in at least 1 diameter by CT scan or MRI as per RECIST 1.1 criteria
- Life expectancy ≥ 3 months
- Patient has adequate bone marrow and organ function
  = Absolute Neutrophil Count (ANC) ≥ 2.0 x 109/L
  = Platelets ≥ 90 x 109/L
  = Hemoglobin ≥ 90 g/L
- Patient has adequate liver function
  = AST and ALT not more than 2.5 times ULN (not more than 5.0 times ULN if there is liver metastasis)
  = Serum bilirubin ≤ 1.2 x ULN
- Creatinine ≤ 1.25 times ULN
- Good compliance

Exclusion Criteria:
- Pregnant or lactating women
- Brain metastasis or only with bone metastasis.
-Patients with severe gastrointestinal hemorrhage which need frequent blood transfusions.
- Refuse to take appropriate contraceptive measures (including male patients).
- Allergic to Oxaliplatin, Irinotecan, Leucovorin or 5-Fluorouracil.
- Severe systemic disease out of control such as unstable or uncompensated respiratory, cardiac, liver, renal diseases.
- Patient has a concurrent malignancy or has a malignancy within 5 years of study enrollment, (with the exception of non-melanoma skin cancer or cervical carcinoma in situ).
- Psychiatric illness that would prevent the patient from giving informed consent.
- Patient is concurrently using other antineoplastic agent
- Patient has used investigational antineoplastic agent within 4 weeks prior to entry.
- Known HIV-positivity.
- No history of chronic diarrhea, nausea or vomit.
- No ≥ grade 2 sensory peripheral neuropathy.
- A history of transmural myocardial infarction (within 6 months prior to entry), congestive heart failure, and unstable angina.
- Infectious disease or inflammation with body temperature ≥ 38 ℃.

Gender	Both
Minimum age	18 Years
Maximum age	65 Years
Healthy volunteers	No

Administrative Data

Organization name	Sun Yat-sen University
Organization study ID	PAN-321
Sponsor	Yuhong Li

Health Authority China: Food and Drug Administration

View of NCT02047474 on 2014_01_27

ClinicalTrials Identifier: NCT02047474
Updated: 2014_01_27

Descriptive Information

Brief title Combination Chemotherapy Before and After Surgery in Treating Patients With Localized Pancreatic Cancer

Official title Phase II Study of Peri-Operative Modified Folfirinox in Localized Pancreatic Cancer

Brief summary

This phase II trial studies how well combination chemotherapy before and after surgery works in treating patients with localized pancreatic cancer. Drugs used in chemotherapy, such as leucovorin calcium, fluorouracil, irinotecan hydrochloride, and oxaliplatin, work in different ways to stop the growth of tumor cells, either by killing the cells or by stopping them from dividing. Giving combination chemotherapy before surgery may make the tumor smaller and reduce the amount of normal tissue that needs to be removed. Giving these treatments after surgery may kill any tumor cells that remain after surgery.

Detailed description

PRIMARY OBJECTIVES:
I. To determine the progression-free survival in patients with resectable non-metastatic pancreatic cancer treated with peri-operative modified leucovorin calcium, fluorouracil, irinotecan hydrochloride, oxaliplatin (mFOLFIRINOX).

SECONDARY OBJECTIVES:
I. Determine overall survival.
II. Determine objective response rate after neoadjuvant mFOLFIRINOX.

TERTIARY OBJECTIVES:
I. Compare R0 resection rate and pathologic stage with institutional historical controls who did not receive neoadjuvant therapy.
II. Correlate early metabolic response, determined by changes in glucose metabolism using positron emission tomography (PET) scanning, with pathologic response, R0 resection, and pathologic stage.
III. Correlate early metabolic response, determined by changes in glucose metabolism using PET scanning, with progression-free and overall survival.
IV. Correlate pre-operative response of CA19-9 with progression-free and overall survival.
V. Collect and bank serial serum and plasma specimens from subjects for future correlative biomarker studies.
VI. Collect and bank tumor tissue from subjects prior to treatment (from the

diagnostic endoscopic ultrasonography [EUS]-guided biopsy) and after treatment with six cycles of FOLFIRINOX (from the surgical specimen) for future correlative biomarker studies.

OUTLINE:
NEOADJUVANT THERAPY: Patients receive mFOLFIRINOX comprising oxaliplatin intravenously (IV) over 2 hours, levoleucovorin calcium IV over 2 hours, irinotecan hydrochloride IV over 90 minutes, and fluorouracil IV continuously for 46 hours on day 1. Treatment repeats every 2 weeks for 6 courses in the absence of disease progression or unacceptable toxicity.

SURGERY: Beginning 3-8 weeks after completion of neoadjuvant therapy patients undergo surgical resection.

ADJUVANT THERARPY: Beginning within 12 weeks after surgery, patients receive mFOLFIRINOX as in neoadjuvant therapy. Treatment repeats every 2 weeks for up to 6 courses in the absence of disease progression or unacceptable toxicity.

After completion of study treatment, patients are followed up every 2 months for 3 years, every 6 months for 2 years, and then annually thereafter.

Phase	Phase 2
Study type	Interventional
Study design	Treatment
Study design	Open Label
Study design	Single Group Assignment
Study design	Efficacy Study
Primary outcome	Measure: Progression free survival rate Time Frame: At 12 months Safety Issue? No Description: Evaluated using a one-sided 0.10-alpha level exact test. Summarized using Kaplan-Meier curves
Secondary outcome	Measure: Overall survival Time Frame: Up to 5 years Safety Issue? No Description: Summarized using Kaplan-Meier curves.
Secondary outcome	Measure: Objective response rate Time Frame: Up to 5 years Safety Issue? No
Enrollment	46 (Anticipated)
Condition	Acinar Cell Adenocarcinoma of the Pancreas
Condition	Duct Cell Adenocarcinoma of the Pancreas
Condition	Stage I Pancreatic Cancer

Condition	Stage IIA Pancreatic Cancer
Condition	Stage IIB Pancreatic Cancer
Arm/Group	Arm Label: Treatment (mFOLFIRINOX) Experimental
	NEOADJUVANT THERAPY: Patients receive mFOLFIRINOX comprising oxaliplatin IV over 2 hours, levoleucovorin calcium IV over 2 hours, irinotecan hydrochloride IV over 90 minutes, and fluorouracil IV continuously for 46 hours on day 1. Treatment repeats every 2 weeks for 6 courses in the absence of disease progression or unacceptable toxicity.
	SURGERY: Beginning 3-8 weeks after completion of neoadjuvant therapy patients undergo surgical resection.
	ADJUVANT THERARPY: Beginning within 12 weeks after surgery, patients receive mFOLFIRINOX as in neoadjuvant therapy. Treatment repeats every 2 weeks for up to 6 courses in the absence of disease progression or unacceptable toxicity.
Intervention	Drug: oxaliplatin Arm Label: Treatment (mFOLFIRINOX)
	Given IV
Intervention	Drug: leucovorin calcium Arm Label: Treatment (mFOLFIRINOX)
	Given IV
Intervention	Drug: irinotecan hydrochloride Arm Label: Treatment (mFOLFIRINOX)
	Given IV
Intervention	Drug: fluorouracil Arm Label: Treatment (mFOLFIRINOX)
	Given IV
Intervention	Procedure/Surgery: therapeutic conventional surgery Arm Label: Treatment (mFOLFIRINOX)
	Undergo surgical resection
Intervention	Other: laboratory biomarker analysis Arm Label: Treatment (mFOLFIRINOX)
	Correlative studies

Recruitment Information

Status	Recruiting
Start date	2013-09

Primary completion date 2015-06 (Anticipated)

Criteria

Inclusion Criteria:

- Pathologic or cytologic documentation of pancreatic adenocarcinoma
- Resectable pancreatic adenocarcinoma disease as defined as follows:
* No evidence of extrapancreatic disease by cross sectional imaging, PET scan, or laparoscopy, including nodal involvement beyond the peripancreatic tissues and/or distant metastases;
* No evidence of tumor extension to superior mesenteric artery, hepatic artery, celiac axis, aorta, or inferior vena cava, and no evidence of occlusion or encasement of the superior mesenteric vein or superior mesenteric vein/portal vein confluence, as assessed by computed tomography (CT) using pancreatic protocol (or magnetic resonance imaging [MRI] in patients who cannot undergo CT) and EUS
- No prior treatment (chemotherapy, biological therapy, or radiotherapy) for resectable pancreatic cancer
- No prior treatment with oxaliplatin, irinotecan (irinotecan hydrochloride), fluorouracil or capecitabine
- Patients who received chemotherapy > 5 years ago for malignancies other than pancreatic cancer are eligible
- There is no evidence of the second malignancy at the time of study entry
- > 4 weeks since major surgery
- No other concurrent anticancer therapy
- Eastern Cooperative Oncology Group (ECOG) performance status: 0-1
- No other malignancy within past five years except basal cell carcinoma of the skin, cervical carcinoma in situ, or non-metastatic prostate cancer
- Paraffin block or slides must be available
- Adequate organ function
- No interstitial pneumonia or extensive and symptomatic interstitial fibrosis of the lung
- No >= grade 2 sensory peripheral neuropathy
- No uncontrolled seizure disorder, active neurological disease, or known central nervous system (CNS) disease
- No significant cardiac disease, including the following: unstable angina, New York Heart Association class II-IV congestive heart failure, myocardial infarction within six months prior to study enrollment
- No history of chronic diarrhea
- Not pregnant and not nursing
- No other medical condition or reason that, in the opinion of the investigator, would preclude study participation
- Absolute neutrophil count >= 1,500/uL
- Platelet count >= 100,000/uL
- Hemoglobin >= 9 g/dL
- Creatinine < 1.5 X upper limit of normal (ULN) or
- Estimated glomerular filtration rate (GFR) > 30 ml/min
- Bilirubin =< 1.5 X ULN
- Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) =< 3 X ULN
- Negative pregnancy test in women of childbearing age

Gender	Both
Minimum age	18 Years
Healthy volunteers	No

Administrative Data

Organization name	Yale University
Organization study ID	1306012255
Secondary ID	NCI-2013-02349 (CTRP (Clinical Trial Reporting Program))
Secondary ID	1306012255 (Yale University)
Secondary ID	P30CA016359 (US NIH Grant Number)
Sponsor	Yale University
Collaborator	National Cancer Institute (NCI)
Health Authority	United States: Federal Government

View of NCT02109341 on 2014_04_08

ClinicalTrials Identifier: NCT02109341
Updated: 2014_04_08

Descriptive Information

Brief title
Phase I/II Study to Evaluate Nab-paclitaxel in Substitution of CPT11 or Oxaliplatin in FOLFIRINOX Schedule as First Line Treatment in Metastatic Pancreatic Cancer

Official title
Phase I/II Study to Evaluate Nab-paclitaxel in Substitution of CPT11 or Oxaliplatin in FOLFIRINOX Schedule as First Line Treatment in Metastatic Pancreatic Cancer

Brief summary

At this moment, FOLFIRINOX is the best treatment for selected patients (pts) with metastatic pancreatic cancer (mPC). Investigator would like to evaluate the substitution of CPT11 or Oxaliplatin in FOLFIRINOX schedule with Nab-paclitaxel (Nab-p) [Nab-FOLFIRI and Nab-FOLFOX].
Doses for Nab-FOLFIRI and Nab-FOLFOX will be determined by the phase I trial. One or both schedules will be evaluated in successive phase II part.

Detailed description

The primary objective for phase I of the study is to determine the MTD of Nab-p when used in substitution of OXA or CPT11 in FOLFIRINOX schedule, as first-line treatment in pts with mPC. The dose finding strategy will be based on the classical 3+3 dose escalation design.

-Analysis sets:
Modified intention-to-treat population: it consists of all pts who are allocated and receive at least one dose of any component of study treatment. Pts will be grouped according to the randomized treatment assignment. Pts treated during the phase I step will be not included in this population.
Safety population: it consists of all pts who are allocated and receive at least one dose of any component of study treatment. Groups are defined by the study treatment actually received. Pts treated at the MTD during the phase I step will be not included in this population.
Statistical methods
Best ORR will be summarized and 95% confidence limits will be calculated according to the exact method for each of the treatment arms included in the phase II step.
All the analyses of primary and secondary efficacy variables will be performed on the modified intention-to-treat population.
The overall incidences of AEs will be summarized. Pts who experienced the same event on more than one occasion are counted only once in the calculation

of the event frequency, at the highest intensity ever observed.
Serious adverse events will be summarized.
All the safety analyses will be performed on the safety population.

-Sample size:
The experimental treatment, to be considered clinically worthwhile, should determine an overall best RR equal to or greater than 40%. According to the Fleming single stage design, for a 90% power towards an alternative hypothesis of an ORR equal to or greater than 40% and a one-sided type I error rate of 5%, respect to the null hypothesis of an ORR equal to or less than 20%, 42 pts must be included in the final evaluation, in each arm of the phase II step. According to the exact binomial test, the experimental treatment will be considered sufficiently promising and candidate to further studies in the case of a major objective response is seen in at least 14 pts.

Phase	Phase 1
Phase	Phase 2
Study type	Interventional
Study design	Treatment
Study design	Non-Randomized
Study design	Open Label
Study design	Parallel Assignment
Study design	Safety/Efficacy Study
Primary outcome	Measure: Dose finding safety and activity Time Frame: 18 months Safety Issue? Yes Description: To determine the maximum tolerated dose (MTD) and dose-limiting toxicities (DLTs) of the combination Nab-paclitaxel+ Irinotecan+ Leucovorin+ 5-Fluorouracil (Nab-FOLFIRI) and of the combination Nab-paclitaxel+ Oxaliplatin+ Leucovorin+ 5-Fluorouracil (Nab- FOLFOX) in pts with mPC in first-line chemotherapy (CT). To assess efficacy of Nab-FOLFIRI and Nab-FOLFOX in pts with mPC in first-line CT , in term of ORR [Complete response (CR) + partial responses (PR)].
Secondary outcome	Measure: Clinical benefit rate [CR+PR+ stable diseases (SD)] Time Frame: 18 months Safety Issue? Yes Description: - Clinical benefit rate [CR+PR+ stable diseases (SD)]: this is defined as the occurrence of either a confirmed CR or PR objective response or a SD over the entire course of treatment, as determined by the RECIST 1.1 criteria based on investigator assessment. Pts for whom no records of

	post-baseline tumor assessments are reported will be counted as non-responders
Secondary outcome	Measure: Progression free survival (PFS) for each schedule Time Frame: 18 months Safety Issue? Yes Description: - Duration of PFS: this is defined as the time between the date of randomization and the date of first evidence of progressive disease or date of death, whichever occurs first. Documentation of disease progression will be defined as per RECIST 1.1 criteria based on investigator assessment. The censoring date for a patient who is known to be progression-free would be the date of the last tumor assessment.
Secondary outcome	Measure: Overall survival (OS), Time Frame: 18 months Safety Issue? Yes Description: - Duration of OS: is the time from the date of randomization to the date of death from any cause. For pts who are still alive on the date of clinical data cut-off for the OS analysis, the last date when the patient is known to be alive on or prior to the clinical cut-off date will be used to determine the censoring date. For pts who do not have any post-baseline information, data will be censored at the date of randomization plus one day.
Secondary outcome	Measure: Quality of life (QoL) for each schedule Time Frame: 18 months Safety Issue? Yes Description: - QoL : assessment of quality of life with the use of the European Organization for Research and Treatment of Cancer (EORTC) quality-of-life core questionnaire (QLQ-C30, version 3.0). The primary QoL endpoint will be the time to a definitive 5% deterioration in the global health status/QoL scale of the QLQ-C30 questionnaire.
Secondary outcome	Measure: Safety profile Time Frame: 18 months Safety Issue? Yes Description: - Safety profile: Safety of the treatment will be evaluated by serious and non serious adverse events (AEs). AEs will be graded according to the CTCAE v4.03
Enrollment	114 (Anticipated)
Condition	Metastatic Pancreatic Cancer
Arm/Group	Arm Label: Nab-FOLFIRI Experimental

	In the phase I study, all pts enrolled in this arm will receive Nab-FOLFIRI: Irinotecan, 180 mg per square meter of body surface area (m2) + Leucovorin, 400 mg/m2 and 5-Fluorouracil, 400 mg/m2 given as a bolus followed by 2400 mg/m2 given as a 46-hour continuous infusion, plus Nab-p per cohort escalation assignment starting with 90 mg/m2 every 2 weeks. Pts continued treatment until a total of 12 administrations, disease progression or unacceptable toxicity. Pts enrolled in arm A for phase II will receive the dose of Nab-FOLFIRI as determined in the Phase I and in the same sequence.
Arm/Group	Arm Label: Nab-FOLFOX Experimental In the phase I study, all pts enrolled in this arm will receive Nab-FOLFOX: Oxaliplatin 85 mg/m2 +Leucovorin, 400 mg/m2 and 5-Fluorouracil, 400 mg/m2 given as a bolus followed by 2400 mg/m2 given as a 46-hour continuous infusion, plus Nab-p per cohort escalation assignment starting with 90 mg/m2, every 2 weeks. Pts continued treatment until a total of 12 administrations, disease progression or unacceptable toxicity. Pts enrolled in arm B for phase II will receive the dose of Nab-FOLFOX as determined in the Phase I and in the same sequence
Intervention	Drug: Paclitaxel bound albumine Arm Label: Nab-FOLFIRI

Recruitment Information

Status	Recruiting
Start date	2014-02
Last follow-up date	2015-07 (Anticipated)
Primary completion date	2014-12 (Anticipated)

Criteria

Inclusion Criteria:
- . Males or females ≥ 18 years old and ≤ 75 years old;
- Histological or cytological evidence of a diagnosis of pancreatic ductal adenocarcinoma;
- Written informed consent prior to any study-specific procedures;
4. Measurable metastatic disease, defined in according to RECIST Version 1.1 (Eisenhower et al. 2009), that had not previously been treated with CT for metastatic disease;
- Eastern Cooperative Oncology Group (ECOG) performance status (PS) score of 0 or 1 ;
- Absence of previous abdominal radiotherapy on target lesions (except radiation therapy analgesic if it has not been performed on measurable targets);

- Absence of heart failure or angina or infarction within 12 months previous inclusion;
- Have adequate organ function including:
Hematologic: absolute neutrophil count (ANC) ≥ 1.5 x 109/L, platelets ≥ 100 x 109/L.
Hepatic: Bilirubin ≤ 1.5 times upper limits of normal (ULN) (Pts may have endoscopic or radiologic stenting to treat biliary obstructions).
Renal: Serum creatinine within normal limits ≤1.5 times ULN.

Exclusion Criteria:
- Age of 76 years or older;
-Endocrine or acinar pancreatic carcinoma;
- Previous radiotherapy for measurable lesions;
- Central nervous system metastasis;
- Other concomitant cancer or history of cancer outside a carcinoma in situ of the cervix or basal or squamous cell of the skin;
- Pts already included in another clinical trial with other experimental drugs;
- Current active infection;
- Have serious pre-existing medical conditions or serious concomitant systemic disorders that would compromise the safety of the patient or his/her ability to complete the study, at the discretion of the investigator (for example, unstable angina pectoris, or a clinically significant history of cardiac disease or uncontrolled diabetes mellitus);
- Females who are pregnant or lactating;
- Unable to undergo medical test for geographical, social or psychological reason
- Known dihydropyrimidine dehydrogenase (DPD) deficiency

Gender	Both
Minimum age	18 Years
Maximum age	75 Years
Healthy volunteers	No

Administrative Data

Organization name	Gruppo Oncologico Italiano di Ricerca Clinica
Organization study ID	Goirc 01-2013
Secondary ID	2013-002275-18 (EudraCT Number)
Sponsor	Gruppo Oncologico Italiano di Ricerca Clinica
Health Authority	Italy: The Italian Medicines Agency

View of NCT02143219 on 2014_05_20

ClinicalTrials Identifier: NCT02143219
Updated: 2014_05_20

Descriptive Information

Brief title
Efficacy and Tolerance Evaluation in FOLFIRINOX Dose Adjusted in Elderly Patients With a Metastatic Pancreatic Cancer

Official title
Phase-2 Study Evaluating Overall Response Rate (Efficacy) and Autonomy Daily Living Preservation (Tolerance) of "FOLFIRINOX " Pharmacogenetic Dose Adjusted, in Elderly Patients (70 yo. or Older) With a Metastatic Pancreatic Adenocarcinoma.

Brief summary

Metastatic pancreatic carcinomas represent the 5th cause of cancer death in France (#8000 per year). The median age at diagnosis is 69 and 74 in male and female respectively. When the 5-Fluorouracile has been used as a single agent with a limited efficacy during more than 20 years, the onset of gemcitabine in 1995 has led to a moderate increase of median survival (from 4.41 to 5.65 months) and overall survival at 1 year (2 versus 18%). Recently, in a phase II followed by a phase-III study, a French collaborative group has demonstrated the benefit of "FOLFIRINOX " regimen versus gemcitabine alone, in terms of median survival (11.1 versus 6.8 months), progression-free survival (6.4 versus 3.3 months) and response rate (31.6 versus 9.4%).
Although more hematologic (neutropenia) and GI toxicities were observed, FOLFIRINOX was acceptable as a new standard regimen for the majority of patients under the age of 70 with a good Performans Status. To reduce the toxicity of FOLFIRINOX in elderly patients (> 70 yo), pharmacogenetic monitoring of 5-FU and Irinotecan key metabolism enzymes (DPD and UGTA1) may be easily performed. The methodology of the study is to use the Bryant & Day statistical method, allowing to consider simultaneously as principal objective, the response rate (efficacy) and the tolerance (preservation of autonomy daily living, Katz index): this design is particularly fitting in a study for elderly patients who represent half of the pancreatic carcinoma population.

Detailed description

METHODOLOGY :
Phase II study, opened, multicentric

MAIN OBJECTIVE :
The main objective is the simultaneous evaluation of the objective rate of answer and toxicity of her(it) of the protocol FOLFIRINOX administered to doses

adapted at patients of 70 and more years old.

SECONDARY OBJECTIVE :
· Efficiency evaluation;
· Tolerance evaluation;
· Quality of Life (QoL) and clinical profit.

STATISTICAL ANALYSIS:
An analysis in two stages is planned, according to the method of Bryant and Day with a risk ß 5 % to reject wrongly an effective treatment and of acceptable toxicity and a risk a=10 % to accept wrongly a not rather effective or too toxic treatment.
The study will be considered as successful if:
· we obtain at least 11 tumoral answers and
· maxi 30 patients on 72 are in loss of autonomy (decrease of their ADL).
=> All the patients who will have received at least an injection will be eligible for the evaluation of the toxicity
=> The evaluation of the efficiency will be made after 3 cures at least unless early termination where the scanner will be anticipated.
=> All the toxicity will be increased according to criteria of toxicity NCI-CTC v4.0.
=> The evaluation of the tumoral answer (CR, PR and SD) will be made according to the criteria RECIST-v1.1.

Phase	Phase 2
Study type	Interventional
Study design	Treatment
Study design	Open Label
Study design	Single Group Assignment
Study design	Safety/Efficacy Study
Primary outcome	Measure: 1st step analysis : Safety and efficacy after 34 patients included Time Frame: 12 weeks after the 34th patient included Safety Issue? Yes Description: Evaluation of Efficacy: Progression-free-Survival (PFS) and Overall Survival (OS) will be evaluated. Evaluation of Toxicity: Will be analyzed, according to the NCI-CTCAE version 4.0: • The incidence of hematological toxicities (grade 3-4, in particular neutropenia and febrile neutropenia) • The incidence of GI toxicities, in particular diarrhea and oral mucositis • The incidence of peripheral neuropathies For statistical analysis : either >= 17 patients show a decrease of their ADL (of 1.5 ADL or more) : the treatment is considered as being too toxic,

	either <= 3 patients presented a tumoral response: the treatment is considered as not being effective enough, => The study will then be arrested in this 1st stage.
Secondary outcome	Measure: 2nd step analysis : Safety and efficacy after 72patients included Time Frame: 12 weeks after the 72th patient included Safety Issue? Yes Description: Only if 1st step is successful we can do the second step : . For toxicity : if >= 31patients show a decrease of their ADL (of 1.5 ADL or more) and/or . For efficacy : if <= 10 patients presented a tumoral response => Study is successful if : . we obtain at least 11 tumoral response and . maximum 30 patients on 72 evaluable are in loss of autonomy (ADL)
Enrollment	72 (Anticipated)
Condition	Pancreatic Metastatic Cancer
Condition	Toxicity
Arm/Group	Arm Label: FOLFIRINOX Other FOLFIRINOX (D1-D15, for maximum 12 cycles) = Oxaliplatine + Folinic acid + Irinotecan + 5-FU
Intervention	Drug: Oxaliplatine Arm Label: FOLFIRINOX Oxaliplatine : 85mg/m², 2-hours IV infusion (D1), then,
Intervention	Drug: Folinic acid Arm Label: FOLFIRINOX Folinic acid (FA): 400 mg/m² , 2-hour IV infusion (D1),
Intervention	Drug: Irinotecan Arm Label: FOLFIRINOX Irinotecan (at the dosage determined by the UGT1A1 status), 90 min IV infusion starting 30 min after the FA starts • Homozygous 6/6 or 6/7: irinotecan will start at 150 mg/m², then will be increased according to clinical/biological tolerance by 10% steps, at each cycle, up to 180 mg/m² at max. • Homozygous 7/7: irinotecan will start at 130 mg/m² in the first cycle then be increased up to a max of 150 mg/m², by 10% steps, according to tolerance.
Intervention	Drug: 5-FU Arm Label: FOLFIRINOX 5-FU (according to the DPD pharmacogenetic status), continuous IV infusion of 46 hours, starting at the end of FA infusion:

• If no DPD deficiency, 5-FU start at 1600 mg/m² and can be modulated according to clinical/biological tolerance after each course, i.e., 1800 mg/m² the 2nd course and 2000 mg/m² the 3rd one
• If partial DPD deficiency: 5-FU start at 1200 mg/m² and can be increased up to 1800, then 2000 if the clinical/biological tolerance are good at the 2nd and 3rd course.

Recruitment Information

Status	Not yet recruiting
Start date	2014-05
Last follow-up date	2021-11 (Anticipated)
Primary completion date	2016-06 (Anticipated)

Criteria

Inclusion Criteria:
- Histologically proven ductal pancreatic carcinoma
- Metastatic disease
- First-line treatment : No previous chemotherapy in metastatic stage but adjuvant treatment before relapse (secondary metastatic) is permitted, provide it has been administered more than 6 months before)
- Age of 70 yo or above
- Normal DPD enzyme level or partial defect (excluding total defect)
- Adequate bone marrow reserve: as indicated by : neutrophils >1500/mm3, platelets >100,000/ mm3, Hb >10.0g/dL.
- Adequate Renal function as indicated by: MDRD creatinine clearance > 50ml/min.
- Adequate hepatic function as indicated by: serum bilirubin < 1.5 times the upper limit of normal, AST and ALT < 2.5 times the upper limit of normal, or < 5 times the upper limit of normal if liver metastases are present.
- Written informed consent must be obtained prior to protocol-specific procedures are being performed
- Patient is affiliated to a social security category

Exclusion Criteria:
- Other than ductal pancreatic carcinoma: namely endocrin tumors, acinar cells carcinoma, cystadenocarcinoma or adenocarcinoma of the ampulla of vater
- Non-metastatic but locally advanced pancreatic adenocarcinoma
- Complete DPD deficiency
- History of Cardiac failure or symptomatic coronary artery disease
- Autonomy Daily Living score by Katz <4
- Prior treatment with FOLFIRINOX (adjuvant)
- Major comorbidity likely to be an obstacle to treatment
- Active or uncontrolled infection such as HIV or chronic B or C hepatitis
- Uncontrolled diabetes mellitus
- Prior peripheral neuropathy, grade > 2
- Inflammatory bowel disease localized on the colon or rectum; bowel

obstruction or severe uncontrolled diarrhea
- Previous or concomitant malignancies other than effectively treated carcinoma in situ of the cervix or non-melanoma skin cancer
- Hereditary fructose intolerance
- Persons deprived of liberty or under guardianship
- Any social, geographical or psychological condition which would compromise the ability to fully comply with the trial procedures and treatments

Gender	Both
Minimum age	70 Years
Healthy volunteers	No

Administrative Data

Organization name	Institut Cancerologie de l'Ouest
Organization study ID	ICO-N-2014-01
Secondary ID	2014-000539-17 (EudraCT Number)
Sponsor	Institut Cancerologie de l'Ouest
Health Authority	France: Agence Nationale de Sécurité du Médicament et des produits de santé

View of NCT02148549 on 2014_05_27

ClinicalTrials Identifier: NCT02148549
Updated: 2014_05_27

Descriptive Information

Brief title Neoadjuvant FIRINOX for Borderline Resectable Pancreatic Cancer - a Pilot Study

Official title The Pilot Study of Neoadjuvant Chemotherapy of FIRINOX for Patients With Borderline Resectable Pancreatic Cancer

Brief summary

FOLFIRINOX regimen was recently presented at an international oncology meeting and represents a new standard regimen in the treatment of metastatic pancreatic cancer. FOLFIRINOX is one of the high response rate treatment regimen , the investigators considered as a promising treatment as neoadjuvant chemotherapy . On the other hand , incidences of grade 3 or 4 neutropenia , febrile neutropenia and diarrhea were significantly higher in the FOLFIRINOX group compared with gemcitabine group. Therefore, it was decided to consider the balance of safety and efficacy as a preoperative chemotherapy, the investigators use the FIRINOX regimen by eliminating LV and bolus 5-FU, and irinotecan reduced to 150mg/m2 of 180mg/m2 from FOLFIRINOX regimen

Detailed description

FOLFIRINOX regimen was recently presented at an international oncology meeting and represents a new standard regimen in the treatment of metastatic pancreatic cancer. FOLFIRINOX is one of the high response rate treatment regimen , the investigators considered as a promising treatment as neoadjuvant chemotherapy . On the other hand , incidences of grade 3 or 4 neutropenia , febrile neutropenia and diarrhea were significantly higher in the FOLFIRINOX group compared with gemcitabine group. Therefore, it was decided to consider the balance of safety and efficacy as a preoperative chemotherapy, the investigators use the FIRINOX regimen by eliminating LV and bolus 5-FU, and irinotecan reduced to 150mg/m2 of 180mg/m2 from FOLFIRINOX regimen. The investigators also evaluate the optimal treatment schedule of FIRINOX therapy as neoadjuvant chemotherapy, optimal duration between surgery and chemotherapy, R0 resection rate, and resection rate for borderline resectable pancreatic cancer.

Phase Phase 1
Study type Interventional
Study design Treatment
Study design Open Label

Study design	Single Group Assignment
Study design	Safety/Efficacy Study
Primary outcome	Measure: Number of participants with toxicity of FIRINOX therapy as neoadjuvant chemotherapy for borderline resectable pancreatic cancer. Time Frame: Up to 30 weeks. Safety Issue? No Description: Toxicities will be assessed according to Common Terminology Criteria for Adverse Events (CTCAE) 4.0.
Secondary outcome	Measure: The resection rate of FIRINOX therapy as neoadjuvant chemotherapy for borderline resectable pancreatic cancer. Time Frame: Up to 24 weeks. Safety Issue? No
Secondary outcome	Measure: The R0 resection rate of FIRINOX therapy as neoadjuvant chemotherapy for borderline resectable pancreatic cancer. Time Frame: Up to 30 weeks. Safety Issue? No
Secondary outcome	Measure: The optimal treatment schedule of FIRINOX therapy as neoadjuvant chemotherapy for borderline resectable pancreatic cancer. Time Frame: Up to 2 years. Safety Issue? No
Enrollment	10 (Anticipated)
Condition	Pancreatic Cancer.
Arm/Group	Arm Label: Optimal chemotherapy courses Experimental Neoadjuvant chemotherapy 4 courses of FIRINOX early 5 patients, and 8 courses of FIRINOX subsequent 5 patients
Intervention	Drug: FIRINOX Arm Label: Optimal chemotherapy courses FIRINOX regimen by eliminating LV and bolus 5-FU, and irinotecan reduced to 150mg/m2 of 180mg/m2 from FOLFIRINOX regimen.

Recruitment Information

Status	Recruiting
Start date	2014-04
Last follow-up date	2017-02 (Anticipated)
Primary completion date	2017-02 (Anticipated)

Criteria

Inclusion Criteria:
• Pathologically proven invasive pancreatic ductal carcinoma
• Cases that meet the definition of borderline resectable pancreatic cancer 1) or 2)
1) Definition of a borderline resectable pancreatic cancer is filledin NCCN guideline version 1.2014 pancreatic adenocarcinoma
2) Patients indicated distal pancreatectomy with en bloc celiac axis resection
• PS (ECOG) 0-1
• ≧20 years old and < 75 years old
• First line treatment
• The following criteria must be satisfied in laboratory tests within 14 days of registration
White blood cell count ≦12,000/mm3
Neutrophil count ≧1,500/mm3
Platelet count ≧100,000mm3
Total bilirubin < 2.0mg/dL
Serum Creatinine ≦upper limits of normal(ULN)
AST, ALT≦2.5×ULN
Albumin≧3.0g/dL
Hemoglobin≧9.0g/dL
• Written informed consent to participate in this study

Exclusion Criteria:
• Severe drug hypersensitivity
• Multiple primary cancers within 5 years
• Severe infection
• With grade2 or more severe peripheral neuropathy
• With intestinal paralysys, ileus
• Interstitial pneumonia or pulmonary
• With uncontrollable pleural effusion or ascites
• Receiving atazanavir sulfate
• With uncontrollable diabetes
• With uncontrollable heart failure, angina, hypertension, arrhythmia
• With severe psychological symptoms
• With watery diarrhea
• Pregnant or lactating women, or women with known or suspected pregnancy
• Inappropriate patients for entry on this study in the judgment of the investigator
• With UGT1A1*28 and/or UGT1A1*6 polymorphisms

Gender	Both
Minimum age	20 Years
Maximum age	74 Years
Healthy volunteers	No

Administrative Data

Organization name	Wakayama Medical University

Organization study ID	FIRINOX
Secondary ID	UMIN000013809 (UMIN)
Sponsor	Wakayama Medical University
Health Authority	Japan: Ministry of Health, Labor and Welfare

View of NCT02896803 on 2016_09_11

ClinicalTrials Identifier: NCT02896803
Updated: 2016_09_11

Descriptive Information

Brief title
Fluorouracil and Oxaliplatin as First-line for Advanced Pancreatic Cancer

Official title
A Phase II Trial of Bolus Fluorouracil and Oxaliplatin (mFLOX) as First-line Regimen for Patients With Unresectable or Metastatic Pancreatic Cancer Not Eligible for Infusional Fluorouracil, Irinotecan and Oxaliplatin

Brief summary

Patients with locally advanced or metastatic pancreatic adenocarcinoma not eligible for infusional fluorouracil, irinotecan and oxaliplatin (FOLFIRINOX) (PPS 2 or hyperbilirubinemia, among other causes) will be treated with mFLOX regimen (fluorouracil bolus and oxaliplatin). The primary endpoint is to assess the objective response rate according to RECIST criteria (version 1.1) and the secondary endpoints are time until clinical or radiological progression, overall survival, toxicity profile.

Detailed description

Currently, FOLFIRINOX is considered the standard treatment for PS 0 or 1 patients with advanced pancreatic carcinoma. However, due to excessive toxicity dose reductions and interruptions in the treatment toxicity are frequent. So, for those not eligible patients (PS 2 or 3, hyperbilirubinemia, among other causes), alternative schemes as gemcitabine alone are the standard approach .
This study aims to evaluate the efficacy and safety of the mFLOX regimen (fluorouracil bolus and oxaliplatin) as first-line regimen for advanced pancreatic adenocarcinoma not eligible for FOLFIRINOX.
The primary endpoint is to assess the objective response rate according to RECIST criteria (version 1.1) and the secondary endpoints are time until clinical or radiological progression, overall survival, toxicity profile.
It has been estimated an n=34 for a response rate of 20%, compared to the historical control of 7% with gemcitabine alone (Von Hoff et al.), with an alpha error of 5% and power of 80%. Considering a rate of 10% of dropout, our sample will be 37 patients.

Phase Phase 2
Study type Interventional
Study design Treatment
Study design Open Label
Study design Single Group Assignment

Study design	Efficacy Study
Primary outcome	Measure: Response rate Time Frame: Through the study, every 14-16 weeks, until an average of 6 months Safety Issue? No Description: Response rate will be evaluated according RECIST criteria version 1.1
Secondary outcome	Measure: Time to progression Time Frame: Through the study, every 14-16 weeks, until an average of 6 months Safety Issue? No Description: CT scans will be performed every 14-16 weeks, until disease progression (according to RECIST criteria version 1.1) or death, an average of 6 months.
Secondary outcome	Measure: Overall survival Time Frame: Through the study, an average of 10 months Safety Issue? No Description: It is defined as a time between entry in the trial and death
Secondary outcome	Measure: Toxicities according CTCAE v4.03 Time Frame: Through the treatment, every visit, an average of 6 months Safety Issue? Yes Description: Toxicities will be evaluated every visit, according CTCAE v4.03
Enrollment	37 (Anticipated)
Condition	Pancreatic Neoplasms
Arm/Group	Arm Label: Experimental Experimental mFLOX
Intervention	Drug: mFLOX Arm Label: Experimental 5-fluorouracil 500 mg/m2 and folinic acid 20 mg/m2 infused both bolus weekly for 6 weeks (d1, 8,15, 22, 29 and 36) and oxaliplatin 85 mg / m2 infused over 2 hours at weeks 1,3 and 5 (d1,15 and 29). The scheme will be repeated every 8 weeks.

Recruitment Information

Status	Recruiting
Start date	2016-08

Last follow-up date 2018-08 (Anticipated)
Primary completion date 2018-08 (Anticipated)
Criteria

Inclusion Criteria:
- Patients with pancreatic adenocarcinoma, confirmed by biopsy and histological material available for review
- Unresectable primary tumor considered by the team assistant or metastatic disease
- Aged between 18 and 75 at the time of study entry
- Naïve patients of palliative chemotherapy, admitted for treatment at the Institute of the São Paulo State Cancer (ICESP)
- Patients with performance status 0 or 1, not candidates to receive chemotherapy with FOLFIRINOX or performance status 2.
- No significant organ dysfunction defined as: Hb> 9 g / dL, platelets> 100,000 / microliter (mcL), neutrophils> 1500 / mcL, clearance of creatinine (ClCr) > 50 ml / min, total bilirubin <5 mg/dl, serum alanine transaminase (ALT) and aspartate transaminase (AST) <2.5 x upper limit of normal (ULN) (or <5 x ULN if liver metastases present)
- Able to read and sign an informed consent form.

Exclusion Criteria:
- Use of prior chemotherapy with other agents, except adjuvant chemotherapy with gemcitabine monotherapy since completed more than 6 months
- Absence of histological material available to local review (eg diagnostic fine needle aspiration (FNA) or cytology)
- Previous use of radiotherapy in the primary tumor or a metastasis site that will serve as target lesion to assess response to treatment
- Diagnosis of malignancy other activity except non-melanoma skin cancer
- Clinical evidence of metastasis in the central nervous system active meningeal carcinomatosis or
severe chronic disease patients (cirrhosis, heart failure New York Heart Association Functional Classification (NYHA) III or IV, chronic obstructive pulmonary disease (COPD) oxygen-dependent or chronic kidney disease requiring dialysis)
- Pregnant or breastfeeding
- Patients with HIV / AIDS story on anti-retroviral therapy
- Patients with peripheral neuropathy grade> 2 (CTCAE v4.03)
- Medium or large surgery in the last 4 weeks. For example, biliary derivation.

Gender Both
Minimum age 18 Years
Maximum age 75 Years
Healthy volunteers No

Administrative Data

Organization name Instituto do Cancer do Estado de São Paulo
Organization study ID 869/15
Sponsor Instituto do Cancer do Estado de São Paulo

Health Authority Brazil: Ethics Committee

View of NCT02896907 on 2016_09_11

ClinicalTrials Identifier: NCT02896907
Updated: 2016_09_11

Descriptive Information

Brief title Ascorbic Acid and Combination Chemotherapy in Treating Patients With Locally Advanced or Recurrent Pancreatic Cancer That Cannot Be Removed by Surgery

Official title A Pilot Study of Intravenous Ascorbic Acid and Folfirinox in the Treatment of Advanced Pancreatic Cancer

Brief summary

This pilot clinical trial studies the side effects of ascorbic acid and combination chemotherapy in treating patients with pancreatic cancer that has spread to other places in the body, has come back, or cannot be removed by surgery. Nutrients found in food and dietary supplements, such as ascorbic acid, may improve the tolerability of chemotherapy regimens. Drugs used in chemotherapy, such as fluorouracil, irinotecan hydrochloride, and oxaliplatin, work in different ways to stop the growth of tumor cells, either by killing the cells, by stopping them from dividing, or by stopping them from spreading. Giving ascorbic acid and combination chemotherapy may work better in treating patients with pancreatic cancer.

Detailed description

PRIMARY OBJECTIVES:
I. To determine safety of intravenous ascorbic acid in combination with fluorouracil, irinotecan hydrochloride, leucovorin calcium, and oxaliplatin (FOLFIRINOX) as defined by Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 in patients with advanced pancreatic cancer.

SECONDARY OBJECTIVES:
I. To test feasibility of collecting quality of life (QOL), patient reported outcomes (PRO) data and correlative studies on patients with advanced pancreatic cancer.

Phase N/A
Study type Interventional
Study design Treatment
Study design Open Label
Study design Single Group Assignment
Study design Safety Study
Primary outcome

	Measure: Incidence of adverse events as determined by CTCAE version 4.03 Time Frame: Up to 28 days after the last treatment Safety Issue? Yes Description: After 4 patients are enrolled on the study and receive at least one dose of intravenous ascorbic acid, the data will be reviewed. If 2 out of the 4 cannot complete 2 courses of FOLFIRINOX then the study will be halted.
Secondary outcome	Measure: Change in quality of life as defined by European Organization for Research and Treatment of Cancer Quality of Life Questionnaire C-30 Time Frame: Baseline to up to 28 days after the last treatment Safety Issue? No Description: Change in quality of life over the six measurement times will be modeled using mixed effects linear regression to account for correlation among repeated measurements from the same subjects. Average change in QoL from baseline to follow-up will be computed.
Enrollment	8 (Anticipated)
Condition	Pancreatic Adenocarcinoma
Condition	Recurrent Pancreatic Carcinoma
Condition	Stage III Pancreatic Cancer
Condition	Stage IV Pancreatic Cancer
Condition	Unresectable Pancreatic Carcinoma
Arm/Group	Arm Label: Treatment (FOLFIRINOX, ascorbic acid) Experimental Patients receive oxaliplatin IV over 2 hours, irinotecan hydrochloride IV over 90 minutes, leucovorin calcium IV over 2 hours, and fluorouracil IV continuously over 46 hours on day 1. Patients then receive ascorbic acid IV over 2 hours on days 3, 5, 8, 10 and 12. Courses repeat every 14 days in the absence of disease progression or unacceptable toxicity.
Intervention	Drug: Oxaliplatin Arm Label: Treatment (FOLFIRINOX, ascorbic acid) Given IV
Intervention	Drug: Irinotecan Hydrochloride Arm Label: Treatment (FOLFIRINOX, ascorbic acid) Given IV
Intervention	

	Drug: Leucovorin Calcium (FOLFIRINOX, ascorbic acid) Arm Label: Treatment Given IV
Intervention	Drug: Fluorouracil (FOLFIRINOX, ascorbic acid) Arm Label: Treatment Given IV
Intervention	Dietary Supplement: Ascorbic Acid Arm Label: Treatment (FOLFIRINOX, ascorbic acid) Given IV
URL	http://kimmelcancercenter.org
URL	http://hospitals.jefferson.edu/
See also	Sidney Kimmel Cancer Center at Thomas Jefferson University, an NCI-Designated Cancer Center
See also	Thomas Jefferson University Hospital

Recruitment Information

Status Not yet recruiting

Start date 2016-10

Primary completion date 2020-08 (Anticipated)

Criteria

Inclusion Criteria:

• Capable of giving informed consent
• Histological diagnosis of adenocarcinoma of the pancreas
• Stage IV or recurrent pancreatic cancer by imaging
• Locally advanced unresectable pancreatic cancer by National Comprehensive Cancer Network (NCCN) criteria
• Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0-1
• No prior treatment for metastatic disease (prior neo-adjuvant or adjuvant chemotherapy, except FOLFIRINOX, chemoradiation or radiation allowed)
• White blood count >= 3000
• Platelets >= 100,000
• Total bilirubin =< 1.5 mg/dl
• Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) =< 5 X upper limit of normal (ULN)
• Creatinine < 1.5 mg/dL
• Glucose-6-phosphatase deficiency (G6PD) level of 5-14 units/g hemoglobin (Hgb) or within institutional standard parameters
• All subjects of child producing potential must agree to use contraception or avoidance of pregnancy measures while enrolled on study

Exclusion Criteria:

• Other pancreatic cancer histology (islet cell, acinar, neuroendocrine tumors)

- Resectable pancreatic cancer
- Prior neoadjuvant FOLFIRINOX
- Pregnant or lactating females
- No clinical ascites (mild ascites on scans permissible)
- Central nervous system (CNS) metastasis
- Known congestive heart failure, significant ventricular arrhythmias, cirrhosis, grade 4/5 chronic kidney disease, uncontrolled diabetes
- Active, uncontrolled bacterial, viral, or fungal infection(s) requiring systemic therapy
- Peripheral neuropathy grade 2 or greater
- Any condition, psychiatric or otherwise, that would preclude informed consent, consistent follow-up or compliance with any aspect of the study (e.g., untreated schizophrenia or other significant cognitive impairment, etc.)

Gender	Both
Minimum age	18 Years
Maximum age	75 Years
Healthy volunteers	No

Administrative Data

Organization name	Thomas Jefferson University
Organization study ID	16D.347
Sponsor	Thomas Jefferson University
Health Authority	United States: Food and Drug Administration

A Phase 2, Open-Label Dose-Exploration Study of Liposomal Irinotecan (nal-IRI) Plus 5-Fluorouracil/Leucovorin (5-FU/LV) plus Oxaliplatin (OX) in Patients With Previously Untreated Metastatic Pancreatic Cancer

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BACKGROUND

- The incidence of pancreatic cancer has increased during the past several decades. Globally, it is estimated that over 330,000 new cases of pancreatic cancer are diagnosed, with approximately the same of deaths on an annual basis.[1-3] In 2018, it is estimated that 55,440 patients in the United States will be diagnosed with pancreatic cancer and 44,330 will die.[4]
- Currently, the 5-year survival rate of pancreatic cancer is currently estimated at less than 5%,[3,5] which is projected to become the second-leading cause of cancer-related death by 2030.[6]
- Given the poor prognosis and the low median survival rates of less than 1 year for patients with metastatic disease, new treatment options are still needed, as well as research into novel and predictive biomarkers to help manage the disease.[7,8]
- nal-IRI, a liposomal formulation of irinotecan, has been studied in a randomized, phase 3, international study (NAPOLI-1) in mPAC patients previously treated with gemcitabine-based therapy. In that study, the combination of nal-IRI+5-FU/LV significantly prolonged OS compared with 5-FU/LV treatment alone.[9]
- The current trial-in-progress (*ClinicalTrials.gov*: NCT02551991) is a phase 2, open-label comparative study to assess the safety, tolerability, and efficacy of nal-IRI+5-FU/LV+OX for the first-line treatment of patients with mPAC, as well as to determine phase 3 dosing.

OBJECTIVE

- The objective of this trial is to assess the efficacy and safety of na;-IRI+5-FU/LV+OX, in previously untreated mPAC patients
  - The primary objectives of the current study are to:
    - Evaluate the safety and tolerability of nal-IRI+5-FU/LV+OX
    - Characterize dose-limiting toxicities (DLTs) associated with nal-IRI+5-FU/LV+OX
    - Determine the triplet combination dose of nal-IRI+5-FU/LV+OX for future studies
  - The secondary objectives are to:
    - Characterize the pharmacokinetics of nal-IRI+5-FU/LV+OX
    - Evaluate the clinical efficacy of nal-IRI+5-FU/LV+OX including:
      - Overall response rate (ORR) [complete response (CR) + partial response (PR), per Response Evaluation Criteria in Solid Tumors[30] version 1.1]
      - Disease control rate (DCR) [CR + PR + stable disease (SD), per RECIST v1.1]
      - Best overall response (BOR)
      - Progression-free survival (PFS), and
      - Overall survival (OS)

METHODS

- This open-label, comparative study is being conducted in 6 sites across the United States and Australia, with additional sites being recruited across North America, Europe, Asia-Pacific, and South America.

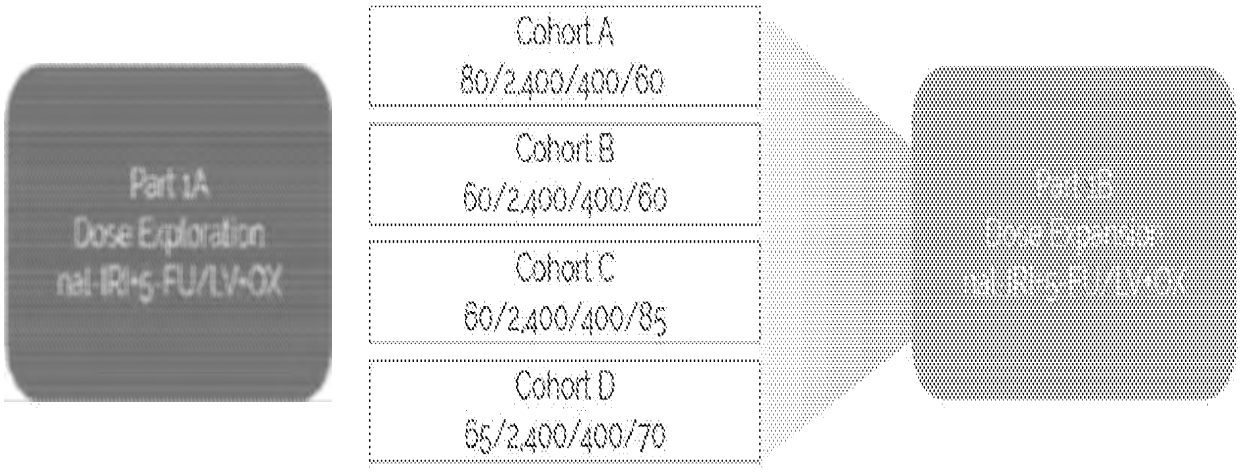
Key Eligibility Criteria

- Adults aged ≥18 years
- Pathologically confirmed, measurable or non-measurable mPAC, as defined by RECIST v1.1, that has not been previously treated in the metastatic setting
  - Unresectable, locally advanced, or metastatic disease is allowed; diagnosed within 6 weeks prior to enrollment
- Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1
- Controlled Central Nervous System metastases
  - Patients who require steroids should be on a stable or decreasing dose
- No history of any second malignancy in the last 3 years
- Adequate hematologic parameters and adequate hepatic (total serum bilirubin ≤ ULN) and renal function (serum creatinine ≤ 1.5 x ULN)
- No clinically significant gastrointestinal disorder, concurrent illnesses, active infection or unexplained fever >38.5°C at screening/baseline, or any other condition deemed likely to interfere with the study

Study Design Overview

- The overall study design is described in Figure 1, with safety and tolerability evaluated across a range of oxaliplatin and nal-IRI dose combinations.

Figure 1. Study Design of the Current nal-IRI+5-FU/LV+OX Trial in Progress



- A dose-exploration safety run-in (traditional 3+3 design) is being performed to confirm an appropriate dose regimen for nal-IRI+5-FU/LV+OX (Table 1).

Table 1. Dose-Exploration Scheme

Cohort	nal-IRI		5-FU/LV		Oxaliplatin	
	Dose (mg/m²)*	Dose day	Dose (mg/m²)	Dose day	Dose (mg/m²)	Dose day
A	80	1,15	2400/400	1,15	60	1,15
B	60	1,15	2400/400	1,15	60	1,15
C	60	1,15	2400/400	1,15	85	1,15
D	65	1,15	2400/400	1,15	70	1,15

*The nal-IRI doses are expressed as the irinotecan HCl trihydrate, whereas doses in the US prescribing information are expressed as the irinotecan free base. Converting the dose is accomplished by substituting the molecular weight of irinotecan HCl trihydrate (677.19 g/mol) with that of irinotecan free base (586.68 g/mol), which results in a conversion factor of 0.866. The above doses of 80 and 60 mg/m² approximate to 70 and 50 mg/m², respectively, based on irinotecan free base.

- DLTs are being assessed during the safety evaluation period.
  - 28 days in Cycle 1
  - 14 days after the 2nd dose of study treatment if there is a treatment delay
- Based on totality of data of all the dose finding cohorts, a dose level will then be selected for the dose expansion cohort, which is intended to enroll n=24 additional patients (a total of N=30 patients for the selected dose level) to obtain additional safety and efficacy data.

Dosing Schedule

- The following dosing schedule is being undertaken during the dose exploration phase
  - Premedication with standard doses of dexamethasone and 5HT3 antagonist or equivalent anti-emetic
  - nal-IRI being administered at a dose range of 60 mg/m² to 85 mg/m² IV over 90 minutes (±10 minutes), on Days 1 and 15 of each 28-day cycle
  - Oxaliplatin being administered at intended dose levels of 60 mg/m² – 85 mg/m² IV over 120 minutes (±10 minutes), on Days 1 and 15 of each 28-day cycle.
  - Leucovorin (l + d racemic form-generic form) being administered at a fixed dose of 400 mg/m², IV over 30 minutes (±5 minutes), on Days 1 and 15 of each 28-day cycle
  - 5-FU being administered at a fixed dose of 2,400 mg/m² IV over 46-hours (±60 minutes), on Days 1 and 15 of each 28-day cycle

Study Evaluations

Safety and Tolerability

- During the dose exploration phase, safety evaluations are conducted regularly by the DLT Committee to review all SAEs, AEs, and DLTs for each patient to determine the safety and tolerability in each Cohort.
- Safety evaluations, including treatment-emergent adverse events (TEAEs) and hospitalization reporting, vital signs, complete blood count, and serum chemistry, are being collected throughout the study.
- TEAEs are being graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE, version 4.03) and are being summarized according to severity and relatedness to treatment.

Efficacy

- All tumor evaluations will be measured according to RECIST v1.1.
- Multiple efficacy measures, including OS, PFS, ORR, BOR, and DCR are assessed in this study

RESULTS

- This analysis provides interim results from 3 of the 4 dose-exploration cohorts of nal-IRI+5-FU/LV+OX that had been initiated by November 10, 2017.
- A total of 24 patients have received ≥1 dose of nal-IRI+5-FU/LV+OX (median age: 66.0 yrs, range: 44–78 yrs) (Table 2).

Table 2. Demographics and Baseline Characteristics: Dose Exploration (Safety Population)

	Cohort A N=7	Cohort B N=7	Cohort C N=10	Total N=24
Gender, n (%)				
Female	6 (85.7%)	4 (57.1%)	2 (20.0%)	12 (50.0%)
Male	1 (14.3%)	3 (42.9%)	8 (80.0%)	12 (50.0%)
Age (years)				
Mean (SD)	66.7 (7.9)	60.4 (10.7)	65.5 (6.2)	64.4 (8.0)
Median	64	57	67	65
Min.–Max.	58–78	44–74	57–73	44–78
Race, n (%)				
Asian	1 (14.3%)	0 (0.0%)	1 (10.0%)	2 (8.3%)
White	6 (85.7%)	7 (100.0%)	9 (90.0%)	22 (91.7%)
ECOG status, n (%)				
0	1 (14.3%)	6 (85.7%)	6 (60.0%)	13 (54.2%)
1	6 (85.7%)	1 (14.3%)	4 (40.0%)	11 (45.8%)
Stage at diagnosis, n (%)				
III	3 (42.9%)	1 (14.3%)	2 (25.0%)	6 (25.0%)
IV	4 (57.1%)	6 (85.7%)	8 (80.0%)	18 (75.0%)

BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; SD, standard deviation

- A total of 5 patients reported ≥1 DLT (2 in Cohort A [1 neutropenic infection and 1 neutropenic sepsis]; 1 in Cohort B [febrile neutropenia], and 2 in Cohort C [diarrhea]).
- The most frequent TEAEs were gastrointestinal disorders (n=24, 100%), including nausea (83.3%), diarrhea (70.8%), vomiting (58.3%), and constipation (45.8%)
- Of the 24 patients treated in the dose exploration phase, n=19 (79.2%) had a treatment-related adverse event that was directly related to treatment with nal-IRI or the triplet combination (Table 3).

Table 3. Incidence of Treatment-Related Treatment Emergent Adverse Events (TRTEAEs) Related to nal-IRI or the Triplet Combination: Dose Exploration Phase (Safety Population, n≥2)*

	Cohort A (n=7	Cohort B (n=7	Cohort C (n=10	Total (N=24
At least 1 TRTEAE	5(71.4%)	7(100.0%)	7(70.0%)	19(79.2%)
Gastrointestinal disorders	5(71.4%)	5(71.4%)	6(60.0%)	16(66.7%)
General disorders and administration site conditions	3(42.9%)	4(57.1%)	4(40.0%)	11(45.8%)
Metabolism and nutrition disorders	5(71.4%)	4(57.1%)	4(40.0%)	13(54.2%)
Blood and lymphatic system disorders	2(28.6%)	3(42.9%)	5(50.0%)	10(41.7%)
Laboratory investigations	1(14.3%)	2(28.6%)	3(30.0%)	6(25.0%)
Skin and subcutaneous tissue disorders	1(14.3%)	3(42.9%)	1(10.0%)	5(20.8%)
Infections and infestations	2(28.6%)	–	1(10.0%)	3(12.5%)
Nervous system disorders	2(28.6%)	–	1(10.0%)	3(12.5%)
Vascular disorders	–	–	3(30.0%)	3(12.5%)
Respiratory, thoracic, and mediastinal disorders	–	1(14.3%)	1(10.0%)	2(8.3%)

*Determination of treatment-related treatment emergent adverse events per investigators' assessment is "related to nal-IRI" or "related to the combination"

- Grade ≥3 TEAEs that were related to nal-IRI or the triplet combination were primarily gastrointestinal disorders (Cohort A: 42.9%; Cohort B: 14.3%; Cohort C: 50.0%) and neutropenia events (neutrophil count decreased, febrile neutropenia, neutropenic infection, neutropenic sepsis; Cohort A: 43%; Cohort B: 29%; Cohort C: 30%) (Table 4).

Table 4. Incidence of Grade ≥3 Treatment Emergent Adverse Events (TEAEs) Related to nal-IRI or the Triplet Combination: Dose Exploration Phase (Safety Population)*

	Cohort A (N=7	Cohort B (N=7	Cohort C (N=13	Total (N=27
At Least 1 Grade ≥3 TEAE	5 (71.4%)	3 (42.9%)	5 (38.5%)	13 (48.1%)
Gastrointestinal disorders	3 (42.9%)	1 (14.3%)	5 (38.5%)	9 (33.3%)
Diarrhea	3 (42.9%)	1 (14.3%)	3 (23.1%)	7 (25.9%)
Vomiting	1 (14.3%)	–	3 (23.1%)	4 (14.8%)
Nausea	–	–	2 (15.4%)	2 (7.4%)
Metabolism and nutrition disorders	3 (42.9%)	2 (28.6%)	2 (15.4%)	7 (25.9%)
Hypokalemia	1 (14.3%)	2 (28.6%)	1 (7.7%)	4 (14.8%)
Decreased appetite	2 (28.6%)	–	–	2 (7.4%)
Dehydration	1 (14.3%)	–	1 (7.7%)	2 (7.4%)
Hypoalbuminemia	1 (14.3%)	–	–	1 (3.7%)
Blood and lymphatic system disorders	1 (14.3%)	2 (28.6%)	2 (15.4%)	5 (18.5%)
Neutropenia	1 (14.3%)	1 (14.3%)	2 (15.4%)	4 (14.8%)
Febrile neutropenia	–	1 (14.3%)	–	1 (3.7%)
Infections and infestations	2 (28.6%)	–	–	2 (7.4%)
Neutropenic infection	1 (14.3%)	–	–	1 (3.7%)
Neutropenic sepsis	1 (14.3%)	–	–	1 (3.7%)
Vascular disorders	–	–	2 (15.4%)	2 (7.4%)
Embolism	–	–	1 (7.7%)	1 (3.7%)
Hypotension	–	–	1 (7.7%)	1 (3.7%)
General disorders and administration site conditions	1 (14.3%)	–	–	1 (3.7%)
Fatigue	1 (14.3%)	–	–	1 (3.7%)
Investigations	–	–	1 (7.7%)	1 (3.7%)
Neutrophil count decreased	–	–	1 (7.7%)	1 (3.7%)
Respiratory, thoracic, and mediastinal disorders	–	–	1 (7.7%)	1 (3.7%)
Pneumonitis	–	–	1 (7.7%)	1 (3.7%)

*Determination of treatment-related treatment emergent adverse events per investigators' assessment i.e. "related to nal-IRI" or "related to the combination"

- The Best Overall Response (BOR) was partial response (PR) in 6 patients (Cohort B: n=3; Cohort C: n=3) (Table 5), with 1 additional patient in Cohort B reported as having achieved PR, but who also underwent surgical resection.

Table 5. Preliminary Summary of Best Overall Response by Investigator Assessment: (Safety Population)

	Cohort A N=7	Cohort B N=7	Cohort C N=10
Best overall response			
Partial response (PR)	–	4* (57.1%)	3 (30.0%)
Stable disease (SD)	3 (42.9%)	2 (28.6%)	1 (10.0%)
Progressive disease (PD)	1 (14.3%)	–	2 (20.0%)
Non-complete response (CR)/Non-PD	1 (14.3%)	–	–
Non-evaluable	2 (28.6%)	1 (14.3%)	4 (40.0%)
Overall Response Rate (CR/PR)	–	4 (57.1%)	3 (30.0%)
Disease Control Rate (CR/PR/SD)	3 (42.9%)	6 (85.7%)	4 (40.0%)

*Includes n=1 patient reported with a PR who underwent surgical resection

- As of 18 May 2018, there are currently 2 patients still receiving treatment with nal-IRI+5-FU/LV+OX. One patient in cohort B, who has completed 22 months of treatment, and one patient in cohort C, who has completed 18 months of treatment.

CONCLUSIONS

- This initial analysis suggests a well-tolerated dose and promising antitumor clinical activity of nal-IRI+5-FU/LV+OX.
- In Cohort B (60/2,400/400/60), which was the lowest and most tolerable dose, 6 of 7 patients reached disease control (PR or SD >16 weeks), with 4 of 7 patients treated for ≥24 weeks.
- Dose exploration and expansion of this trial (*ClinicalTrials.gov*: NCT02551991) is still ongoing.

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A Phase 2, Open-Label Dose-Exploration Study of Liposomal Irinotecan (nal-IRI) Plus 5-Fluorouracil/ Leucovorin (5-FU/LV) plus Oxaliplatin (OX) in Patients With Previously Untreated Metastatic Pancreatic Cancer

[redacted]

BACKGROUND

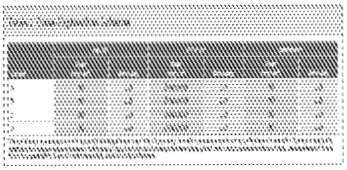
[redacted]

OBJECTIVE

[redacted]

METHODS

[redacted]



RESULTS

[redacted]

CONCLUSIONS

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Poster #TPS-48

A Randomized, Open-label, Phase 2 Study of Nanoliposomal Irinotecan (nal-IRI)–containing Regimens versus nab-Paclitaxel Plus Gemcitabine in Patients with Previously Untreated, Metastatic Pancreatic Adenocarcinoma (mPAC)

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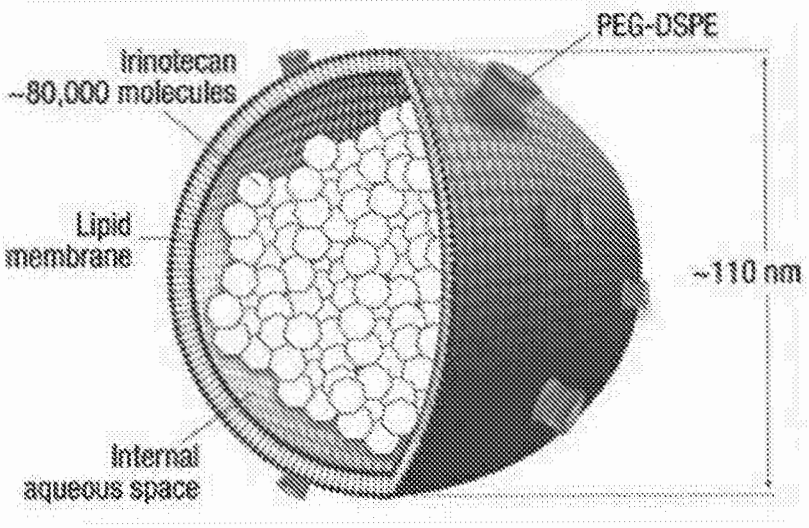
BACKGROUND

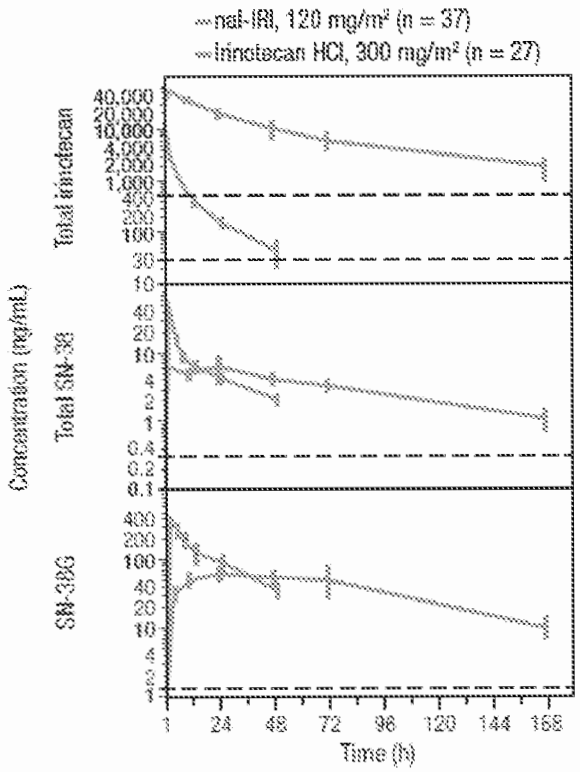
- Two combination chemotherapy regimens have emerged as standard of care for first-line treatment of mPAC:
  - FOLFIRINOX (5-fluorouracil [5-FU] + leucovorin [LV] + irinotecan + oxaliplatin); and
  - nab-paclitaxel + gemcitabine.
- These regimens are associated with median overall survival (OS) of less than one year in Phase 3 studies (11.1 and 8.5 months, respectively).[1,2]
- nal-IRI (ONIVYDE™ [irinotecan liposome injection]; MM-398) is a nanoliposomal formulation of irinotecan, a topoisomerase inhibitor, for intravenous use (**Figure 1**[3]).
  - Pharmacokinetic analyses showed extended circulation of irinotecan within the liposome in patients with gastric cancer treated with nal-IRI at a different dose (120 mg/m^2) and schedule compared with the approved dose and schedule (**Figure 2**).[4,5]
  - The liposome facilitates intratumoral drug deposition through the enhanced permeability and retention effect.[6]
  - Preliminary data from a small pilot study across different cancer types showed higher levels of SN-38 found in tumor biopsies compared with plasma at 72 hours, suggesting local metabolic activation of irinotecan, which was contained in the liposomal nanoparticles, to SN-38 (**Figure 3**).[3]

POSTER PRESENTED AT the Gastrointestinal Cancers Symposium of the American Society of Clinical Oncology; January 21-23, 2016; San Francisco, California.



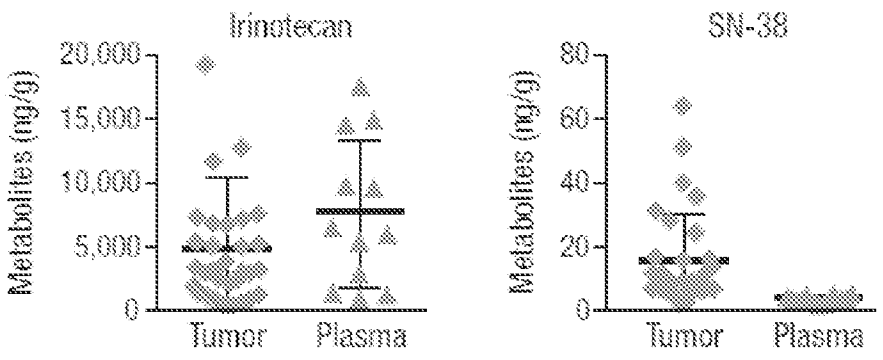
nal-IRI, nanoliposomal irinotecan.

Figure 1. nal-IRI design[2]



nal-IRI, nanoliposomal irinotecan; AUC, area under the curve; C_{max}, maximal concentration.
Comparing nal-IRI with irinotecan HCl, the total irinotecan AUC was 46 times greater and the total irinotecan C_{max} was 13.4 times greater, the SN-38 AUC was 1.4 times greater and the SN-38 C_{max} was 0.19 times greater. The peak of SN-38 metabolite was lower with nal-IRI versus irinotecan HCl, without an increase in SN-38 plasma AUC.

Figure 2. Sustained circulation of nal-IRI[2,3]



nal-IRI, nanoliposomal irinotecan; LLoQ, lower limit of quantification.

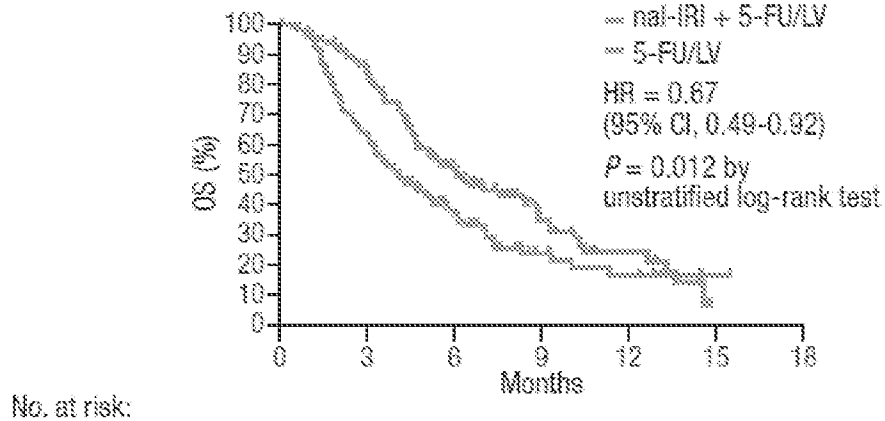
Drug metabolite quantification in tumor biopsies and plasma analyses from a study of patients (N = 14) with advanced solid tumors.

Biopsy material averaged 10.5 mg (range, 3.3-21.9 mg); metabolite detection was in an LC/MS/MS TSQ Vantage instrument, with LLoQ of 50 pg/mL for irinotecan and 100 pg/mL for SN-38.

Plasma analysis was performed at QPS according to validated procedures, with LLoQ of 140 ng/mL for irinotecan and 600 pg/mL for SN-38.

Figure 3. Irinotecan and SN-38 levels 72 hours after nal-IRI treatment[6]

- nal-IRI was recently approved by the US Food and Drug Administration (FDA) for use in combination with 5-FU/LV for the treatment of patients with mPAC after disease progression following gemcitabine-based therapy, based in part on results from the primary analysis of the large (N = 417), international, randomized, Phase 3 study (NAPOLI-1) of nal-IRI in this setting.[7]
  - Median OS increased significantly with nal-IRI + 5-FU/LV relative to 5-FU/LV (6.1 vs 4.2 months; unstratified hazard ratio [HR] = 0.67 [95% confidence interval (CI), 0.49-0.92]; $P = 0.012$; **Figure 4**).
  - Median OS did not differ between patients assigned nal-IRI monotherapy and those allocated to 5-FU/LV (4.9 vs 4.2 months; unstratified HR = 0.99 [95% CI, 0.77-1.28]; $P = 0.94$).
  - Median progression-free survival (PFS; 3.1 vs 1.5 months; unstratified HR = 0.56 [95% CI, 0.41-0.75]; $P = 0.0001$) and objective response rate (ORR; 16% vs 1%; $P < 0.0001$) were also improved with nal-IRI + 5-FU/LV compared with 5-FU/LV.
  - nal-IRI + 5-FU/LV demonstrated a predictable and manageable safety profile; the most frequently reported grade ≥3 treatment-emergent adverse events (AEs) were neutropenia, fatigue, diarrhea, and vomiting.



No. at risk:							
nal-IRI + 5-FU/LV:	117	97	51	20	8	0	0
5-FU/LV:	119	68	34	11	6	1	0

OS, overall survival; nal-IRI, nanoliposomal irinotecan; 5-FU, 5-fluorouracil; LV, leucovorin; HR, hazard ratio; CI, confidence interval.
OS analysis includes all patients randomized after implementation of a protocol amendment that added the nal-IRI + 5-FU/LV combination arm.

Figure 4. Kaplan-Meier OS in the NAPOLI-1 trial[7]

STUDY OBJECTIVES

- The overall goal of the current study is to determine the preliminary safety and efficacy of nal-IRI + 5-FU/LV with or without oxaliplatin, as compared with nab-paclitaxel + gemcitabine in previously untreated patients with mPAC (ClinicalTrials.gov identifier: NCT02551991).

Part 1

- The primary objectives of Part 1 are to
  - Evaluate the safety and tolerability of nal-IRI + 5-FU/LV + oxaliplatin; and
  - Characterize dose-limiting toxicities (DLTs) associated with nal-IRI + 5-FU/LV + oxaliplatin and determine the Part 2 triplet combination dose.
- The secondary objective of Part 1 is to characterize the pharmacokinetics of nal-IRI + 5-FU/LV + oxaliplatin.

Part 2

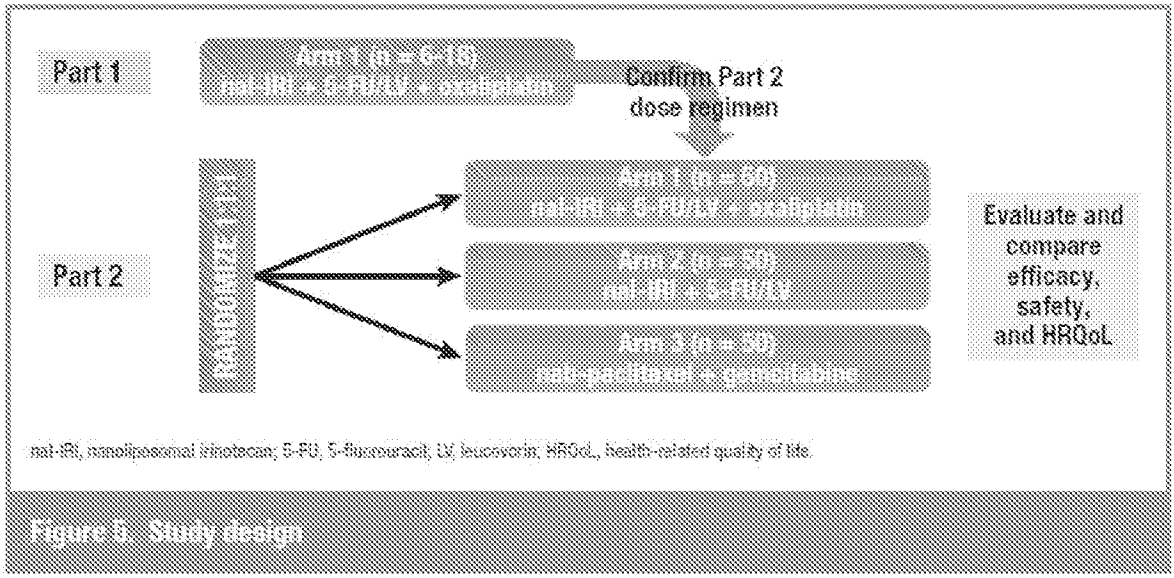
- The primary objective of Part 2 is to compare the PFS rate at 24 weeks in patients in each nal-IRI–containing regimen versus nab-paclitaxel + gemcitabine in first-line mPAC.
- The secondary objectives of Part 2 are to
  - Compare the OS, PFS, and ORR in patients in each nal-IRI–containing regimen relative to nab-paclitaxel + gemcitabine;
  - Assess tumor marker carbohydrate antigen 19-9 (CA19-9) response in patients in each nal-IRI–containing regimen relative to nab-paclitaxel + gemcitabine;
  - Evaluate health-related quality of life (HRQoL) of patients in each treatment arm; and
  - Compare the safety and AE profile between treatment arms.
- An additional exploratory objective is to evaluate patient blood samples and archived tumor tissue for potential biomarkers that may correlate with nal-IRI pharmacokinetics, toxicity, and/or response.

Key Eligibility Criteria

- Adults aged ≥18 years
- Pathologically confirmed, measurable or non-measurable mPAC, as defined by Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1, that has not been previously treated in the metastatic setting
  - Part 1: unresectable, locally advanced or metastatic disease is allowed, diagnosed within 6 weeks prior to enrollment
  - Part 2: must have metastatic disease diagnosed within 6 weeks prior to randomization; locally advanced disease is not allowed
- Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1
- No known metastasis to the central nervous system
- No history of any second malignancy in the last 3 years
- Adequate hematologic parameters, and hepatic and renal function
- Normal electrocardiogram or electrocardiogram without clinically significant findings
- No use of strong CYP3A4 inhibitors or inducers
- No use of strong CYP2C8 inhibitors or inducers (Part 2 only)
- No known contraindications or hypersensitivity to any study drugs (Part 2 only for nab-paclitaxel and gemcitabine)
- No clinically significant gastrointestinal disorder, concurrent illnesses, active infection or unexplained fever >38.5°C at screening/baseline, or any other condition deemed likely to interfere with the study

STUDY DESIGN

- This Phase 2, open-label, comparative study is being conducted in 2 parts (**Figure 5**).



nal-IRI, nanoliposomal irinotecan; 5-FU, 5-fluorouracil; LV, leucovorin; HRQoL, health-related quality of life.

Figure 5. Study design

- Participating sites are located in 15 countries in North America, Europe, Asia-Pacific, and South America (**Figure 6**).

Figure 6. Countries with participating sites

Part 1

- A dose-escalation safety run-in (traditional 3+3 design) is being performed to confirm an appropriate Part 2 dose regimen for the nal-IRI + 5-FU/LV + oxaliplatin triple combination arm (**Table 1**).
  - If no DLTs are reported for a dose cohort within the first treatment cycle, the subsequent cohort will be initiated.
  - If one patient in a dose cohort experiences a DLT within the first treatment cycle, the cohort will be expanded from 3 to 6 patients.
  - If ≥2 patients within a dose cohort experience a DLT, that dose will be considered to exceed the safety criteria and the dose will not be escalated further. The Part 2 dose will then be defined as the next lower dose level in which 6 patients were treated and ≥1 patient experienced a DLT.

Table 1. Dose-escalation Scheme

	Oxaliplatin		5-FU/LV		nal-IRI	
Level	Dose (mg/m²)	Dose day	Dose (mg/m²)	Dose day	Dose (mg/m²)[a]	Dose day
1	60	1, 15	2,400/400	1, 15	80	1, 15
–1	60	1, 15	2,400/400	1, 15	60	1, 15
2	85	1, 15	2,400/400	1, 15	80	1, 15
–2A	75	1, 15	2,400/400	1, 15	80	1, 15
–2B	85	1, 15	2,400/400	1, 15	60	1, 15

5-FU, 5-fluorouracil; LV, leucovorin; nal-IRI, nanoliposomal irinotecan.
Shaded dose levels are for de-escalation only. Enrollment in these dose levels will only be initiated upon agreement of the investigators, study sponsor, and medical monitor.
[a]The above nal-IRI doses are expressed as the irinotecan HCl trihydrate, whereas doses in the US prescribing information are expressed as the irinotecan free base. Converting the dose is accomplished by substituting the molecular weight of irinotecan HCl trihydrate (677.19 g/mol) with that of irinotecan free base (586.68 g/mol), which results in a conversion factor of 0.866. The above doses of 80 and 60 mg/m² approximate to 70 and 52 mg/m², respectively, based on irinotecan free base.

Part 2

- Patients will be randomized (1:1:1) in the active-comparator phase to evaluate and compare efficacy, safety, and HRQoL between treatment arms.
  - Randomization will be stratified based on region (East Asia vs rest of the world) and ECOG PS of 0 versus 1.
  - Part 2 will include the following 3 treatment arms (28-day cycles):
    - Arm 1: nal-IRI + 5-FU/LV + oxaliplatin at dosages determined in Part 1, on Days 1 and 15 of each cycle;
    - Arm 2: nal-IRI (80 mg/m^2 over 90 minutes [70 mg/m^2 based on irinotecan free base]) + 5-FU (2,400 mg/m^2 over 46 hours)/LV (400 mg/m^2) on Days 1 and 15 of each cycle; and
    - Arm 3: nab-paclitaxel (125 mg/m^2 over 35 minutes) + gemcitabine (1,000 mg/m^2 over 30 minutes) on Days 1, 8, and 15 of each cycle.
- Patients will be treated until disease progression, intolerable toxicity, withdrawal of consent, or at the discretion of the treating physician.
- Primary efficacy endpoint: PFS rate at 24 weeks
  - Patients will be considered responders if they have at least one assessment of non–progressive disease, prior to progression or new anticancer therapy, at Week 24 or later.
  - Each nal-IRI–containing arm will be assessed for an increased PFS rate at 24 weeks relative to the control arm using a one-sided Cochran-Mantel-Haenszel test, incorporating randomization stratification factors, at the 0.10 level of significance.
  - If the true PFS rate at 24 weeks in the control arm is 50%, the study would have 78% power to detect an improvement in a nal-IRI–containing experimental arm that has a true rate of 70%.

STUDY POPULATIONS

- Several study populations will be used in the analysis of data (**Table 2**).

Table 2. Study Populations

Population	Description
Safety population	Part 1: Patients receiving any part of ≥1 dose of study drug. This will be the only study population for assessing Part 1 Part 2: Patients receiving any part of ≥1 dose of study drug; this population will be summarized according to treatment actually received
Intent-to-treat population	Part 2 only: All randomized patients; this population will be analyzed according to the randomized treatment arm. This is the primary population for efficacy evaluations
EQ-5D-5L population	Part 2 only: Treated patients who have provided baseline and ≥1 post-baseline assessment for EQ-5D-5L
EORTC-QLQ-C30 population	Part 2 only: Treated patients who have provided baseline and ≥1 post-baseline assessment for EORTC-QLQ-C30
Pharmacokinetic population	nal-IRI–treated patients with ≥1 post–study drug pharmacokinetic assessment

STUDY EVALUATIONS

Efficacy

- Tumor response will be evaluated at least every 8 weeks according to RECIST, version 1.1, based on CT or MRI imaging, until determination of progressive disease.
- CA19-9 levels will be evaluated at least every 8 weeks during treatment and at the end-of-treatment visit. The maximum reduction (percent change from baseline) in CA19-9 will be computed, including analyses by time period.
- Follow-up for survival status will continue at least once every 2 months after treatment discontinuation until death, lost to follow-up, withdrawal of consent, or study closure. OS will be descriptively summarized for each treatment arm using Kaplan-Meier methodology.

Safety and Tolerability

- Safety evaluations, including AE and hospitalization reporting, vital signs, complete blood count, and serum chemistry, will be collected throughout the study.
- AEs will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.03, and will be summarized according to severity and relatedness to treatment.

Quality of Life

- HRQoL will be assessed via the EORTC-QLQ-C30 and EQ-5D-5L instruments at baseline, Day 1 of each subsequent cycle, and at the end-of-treatment visit.

Pharmacokinetics

- Plasma sampling for pharmacokinetic analyses of nal-IRI and oxaliplatin will occur on Days 1, 3, 8, and 15 of Cycle 1 (Parts 1 and 2, Arm 1 only).

Biomarkers

- Analyses will be performed to assess the associations between potential biomarkers (from plasma and archived tissue) and efficacy parameters.
- Whole blood and plasma sampling for biomarker analyses will occur at baseline, Day 1 of each subsequent cycle, and at the end-of-treatment visit.
- Examples of potential analyses include cytokine levels, growth factors, and enzyme levels.

Management of Toxicities

- Guidelines for specific dose modifications are provided in **Table 3** and **Table 4**.
  - Any patient who requires >2 dose reductions or a dose interruption of >2 weeks due to toxicity should be discontinued from study treatment.
  - If oxaliplatin is not well tolerated in Arm 1, the patient may discontinue oxaliplatin and continue to receive nal-IRI + 5-FU/LV.

Table 3. Dose Modification Guidelines for Arms 1 and 2

Grade 3/4 toxicity[a]	nal-IRI	5-FU	Oxaliplatin (Arm 1 only)
Neutropenia or febrile neutropenia	1st occurrence: Reduce to 60 mg/m^2 2nd occurrence: Reduce to 50 mg/m^2	1st occurrence: Reduce dose by 25% 2nd occurrence: Reduce dose by another 25%	*Grade ≥2 events:* 1st occurrence: None 2nd occurrence: Reduce to 60 mg/m^2
Other hematologic toxicity	1st occurrence: Reduce to 60 mg/m^2 2nd occurrence: Reduce to 50 mg/m^2	1st occurrence: Reduce dose by 25% 2nd occurrence: Reduce dose by another 25%	1st occurrence: Reduce to 60 mg/m^2 2nd occurrence: Maintenance of reduced dose at 60 mg/m^2
Hand-foot syndrome	1st occurrence: Reduce to 60 mg/m^2 2nd occurrence: Reduce to 50 mg/m^2	*Grade 2:* 1st occurrence: Reduce dose by 25% 2nd occurrence: Reduce dose by another 25% *Grade 3/4:* Discontinue therapy	None
Neuro-cerebellar toxicity	None	*Any grade:* Discontinue therapy	None
Cardiac toxicity	None	*Grade ≥2 events:* Discontinue therapy	None
Sensory neuropathy	None	None	*Persistent grade 2, or grade 3 that recovers prior to next cycle:* Reduce to 60 mg/m^2 *Persistent grade 3, or grade 4:* Discontinue therapy
Other non-hematologic toxicity[b]	1st occurrence: Reduce to 60 mg/m^2 2nd occurrence: Reduce to 50 mg/m^2	1st occurrence: Reduce dose by 25% 2nd occurrence: Reduce dose by another 25%	1st occurrence: None 2nd occurrence: Reduce to 60 mg/m^2

nal-IRI, nanoliposomal irinotecan; 5-FU, 5-fluorouracil.
Following dose reduction, the patient's dose should remain reduced for the duration of the study.
[a]Grade 3/4 toxicity unless otherwise noted.
[b]For grade 3/4 nausea and vomiting, patients should also receive optimized anti-emetic therapy.

Table 4. Dose Modification Guidelines for Arm 3

Grade 3/4 toxicity[a]	nab-paclitaxel	Gemcitabine
Hematologic or non-hematologic toxicity	1st occurrence: Reduce to 100 mg/m^2 2nd occurrence: Reduce to 75 mg/m^2	1st occurrence: Reduce to 800 mg/m^2 2nd occurrence: Reduce to 600 mg/m^2
Mucositis or diarrhea	Withhold until improves to grade ≤1; reduce dose per Row 1	
Cutaneous toxicity	*Grade ≥2 events:* Reduce dose per Row 1; discontinue therapy if toxicity persists	
Peripheral neuropathy	Withhold until improves to grade ≤1; reduce dose per Row 1	None
Febrile neutropenia	Withhold until fever resolves and ANC ≥1,500/mm^3; reduce dose per Row 1	
Neutropenia or thrombocytopenia	Detailed dose reduction and delay guidelines per administration day and severity of decreased ANC and/or platelet levels	

ANC, absolute neutrophil count.
Following dose reduction, the patient's dose should remain reduced for the duration of the study.
[a]Grade 3/4 toxicity unless otherwise noted.

SUMMARY

- nal-IRI has demonstrated clinical activity and predictable and manageable toxicity in combination with 5-FU/LV in patients with mPAC following gemcitabine-based therapy,[7] leading to its FDA approval in this setting.
- This Phase 2 study will evaluate the safety and efficacy of nal-IRI + 5-FU/LV with or without oxaliplatin versus nab-paclitaxel + gemcitabine in patients with previously untreated mPAC.
- The study may also provide important information on the impact of nal-IRI combination treatment on patient HRQoL and identify potential biomarkers of response.

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A Randomized, Open-Label, Phase 2 Study of Neoadjuvant Inavolisib (GDC-0077) Combined With Paclitaxel and Trastuzumab and Pertuzumab Followed by Inavolisib Plus Trastuzumab and Pertuzumab With Prospectively Untreated, Molecularly Defined Residual Disease After Surgery in Patients With Operable HER2-Positive Breast Cancer: Primary Analysis of the ACERELA (BO41697) Trial

Rugo HS, Im S-A, Cardoso F, Cortés J, Curigliano G, Loi S, Oliveira M, Park YH, Saura C, Wang S, Wildiers H, Zambelli S, Zhang Q, Zhukova L, Bachelot T, Bardia A, Barrios CH, Bianchini G, Chavez-MacGregor M, Chia S, Colleoni M, Conte P, Dalenc F, Delaloge S, Fasching PA, Gianni L, Gnant M, Harbeck N, Huober J, Jerusalem G, Kaufman B, Kim SB, Kim JH, Lee KS, Lu YS, Mahtani R, Mayer IA, Miller K, Morales S, Moreno-Aspitia A, Pistilli B, Rimawi MF, Rodriguez-Lescure Á, Shao Z, Spring L, Stein RC, Swain S, Tolaney SM, Tripathy D, Turner NC, Yamashita T, Yardley DA, Zaman K, Zelnak A, Zhao J, Hyman DM, Armstrong AC, Baselga J, Cristofanilli M, Piccart-Gebhart MJ, Buyse M, Andre F

POSTER PRESENTED AT THE CASTRONTERRA CANCER SYMPOSIUM OF THE AMERICAN SOCIETY OF CLINICAL ONCOLOGY, JANUARY 21-23, 2016; SAN FRANCISCO, CALIFORNIA.

MERRIMACK

Abstract #4830

Preclinical Anti-tumor Activity of Nanoliposomal Irinotecan (nal-IRI, MM-398) + 5-FU + Oxaliplatin in Pancreatic Cancer

Daniel F. Gaddy[1], Helen Lee[1], Nancy Paz[1], Shannon C. Leonard[1], Ashish Kalra1, Jaeyeon Kim1, Ninfa L. Straubinger[2], Charlene M. Thomas[2], Robert M. Straubinger[2], Bryan M. Gillard[3], Michael T. Moser[3], Daryl C. Drummond[1], Stephan G. Klinz[1], Bart S. Hendriks[1], Jonathan B. Fitzgerald[1]

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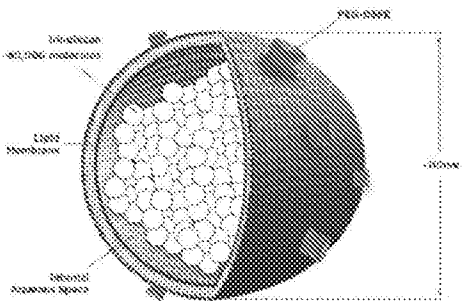
INTRODUCTION

- Pancreatic ductal adenocarcinoma (PDAC) is among the most lethal malignancies world-wide, resulting in more than 330,000 deaths per year [1].
- By 2030, PDAC is predicted to be the second-leading cause of cancer-related mortality in the United States [2].
- Most patients present with advanced disease, including approximately 53% with distant metastases, and another 28% with regional metastases at the time of initial diagnosis [3].
- The FOLFIRINOX regimen (5-fluorouracil/leucovorin (5-FU/LV) + irinotecan/CPT-11 + oxaliplatin) is highly effective in first-line metastatic PDAC, but utilization is limited to patients with good performance scores (ECOG 0-1) due to excessive toxicity [4].
- Nanoliposomal irinotecan (nal-IRI, MM-398) recently gained approval in combination with 5-FU/LV in post-gemcitabine metastatic PDAC.
- Nal-IRI + 5-FU/LV significantly improved overall survival, with a well-defined and manageable toxicity profile, in pretreated patients [5].
- **Here, we evaluate the preclinical anti-tumor activity of a nal-IRI + 5-FU + oxaliplatin regimen relative to a variation of FOLFIRINOX (unencapsulated irinotecan/CPT-11 + 5-FU + oxaliplatin) in support of an ongoing Phase 2 trial (NCT02551991) in first-line metastatic PDAC.**



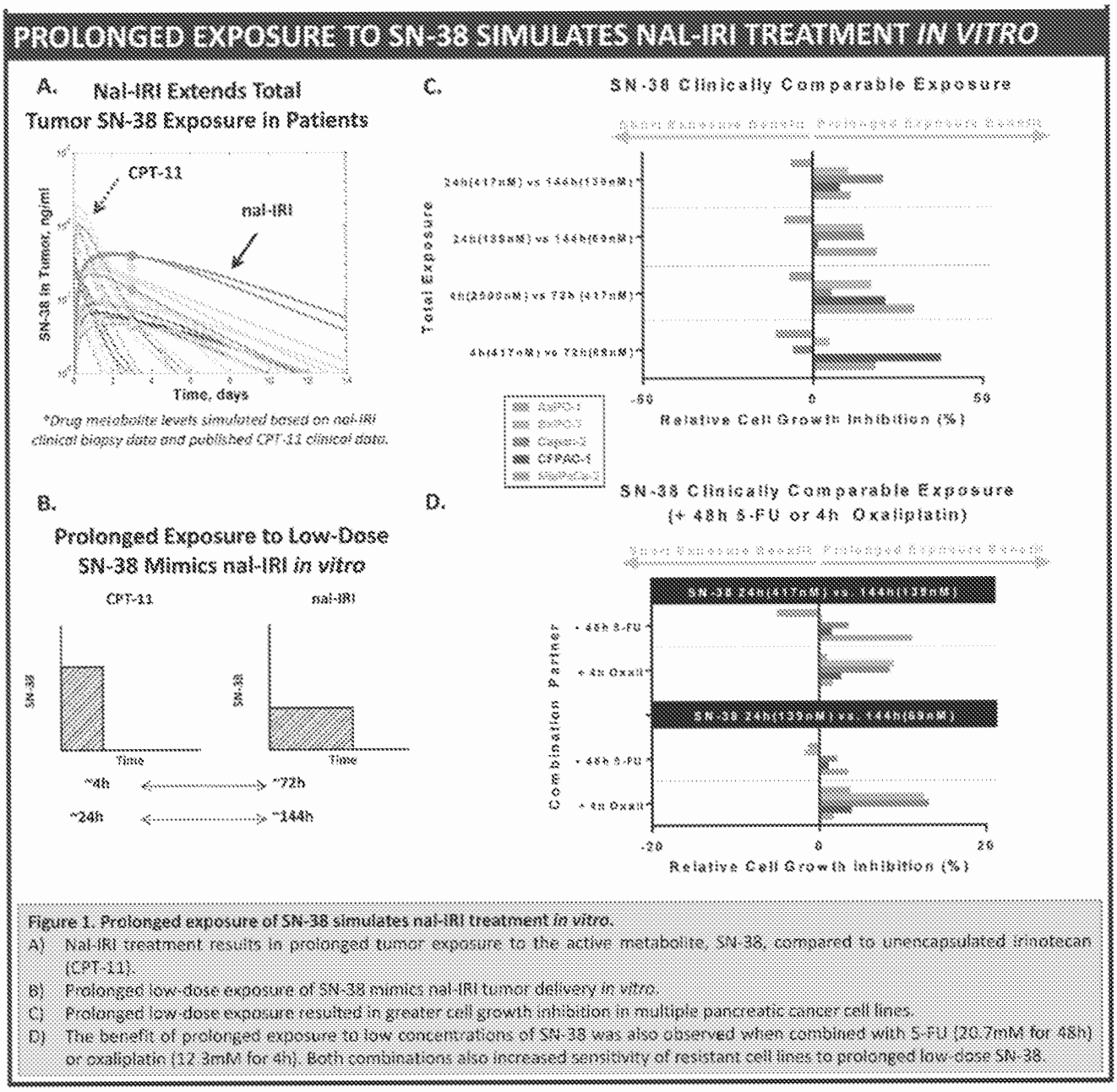


Figure 1. Prolonged exposure of SN-38 simulates nal-IRI treatment *in vitro*.

A) Nal-IRI treatment results in prolonged tumor exposure to the active metabolite, SN-38, compared to unencapsulated irinotecan (CPT-11).

B) Prolonged low-dose exposure of SN-38 mimics nal-IRI tumor delivery *in vitro*.

C) Prolonged low-dose exposure resulted in greater cell growth inhibition in multiple pancreatic cancer cell lines.

D) The benefit of prolonged exposure to low concentrations of SN-38 was also observed when combined with 5-FU (20.7mM for 48h) or oxaliplatin (12.3mM for 4h). Both combinations also increased sensitivity of resistant cell lines to prolonged low-dose SN-38.

NAL-IRI vs CPT-11 PRECLINICAL ACTIVITY WITH 5-FU AND OXALIPLATIN

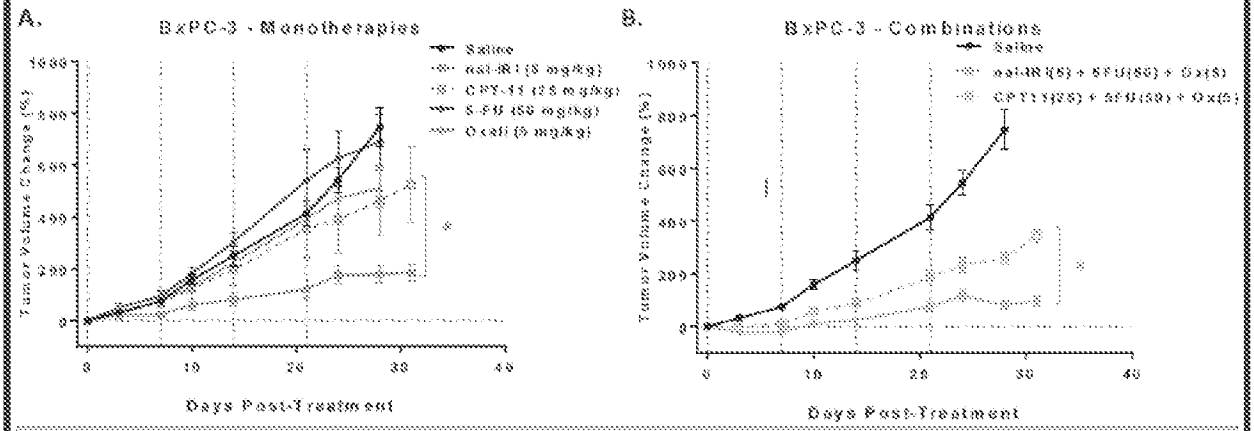


Figure 2. The combination of 5-FU and oxaliplatin with nal-IRI improves response in BxPC-3 pancreatic cancer xenografts.

A) Nal-IRI significantly improved tumor growth control relative to CPT-11. Mice received 8 weekly doses of each agent (vertical dotted lines). All dosages are based on the hydrochloride salt form of irinotecan.

B) The addition of 5-FU and oxaliplatin to both CPT-11 and nal-IRI significantly improved tumor growth control relative to the respective monotherapies. Moreover, the combination with nal-IRI was significantly more active than the combination with CPT-11. Significance determined by ordinary 2 way ANOVA.

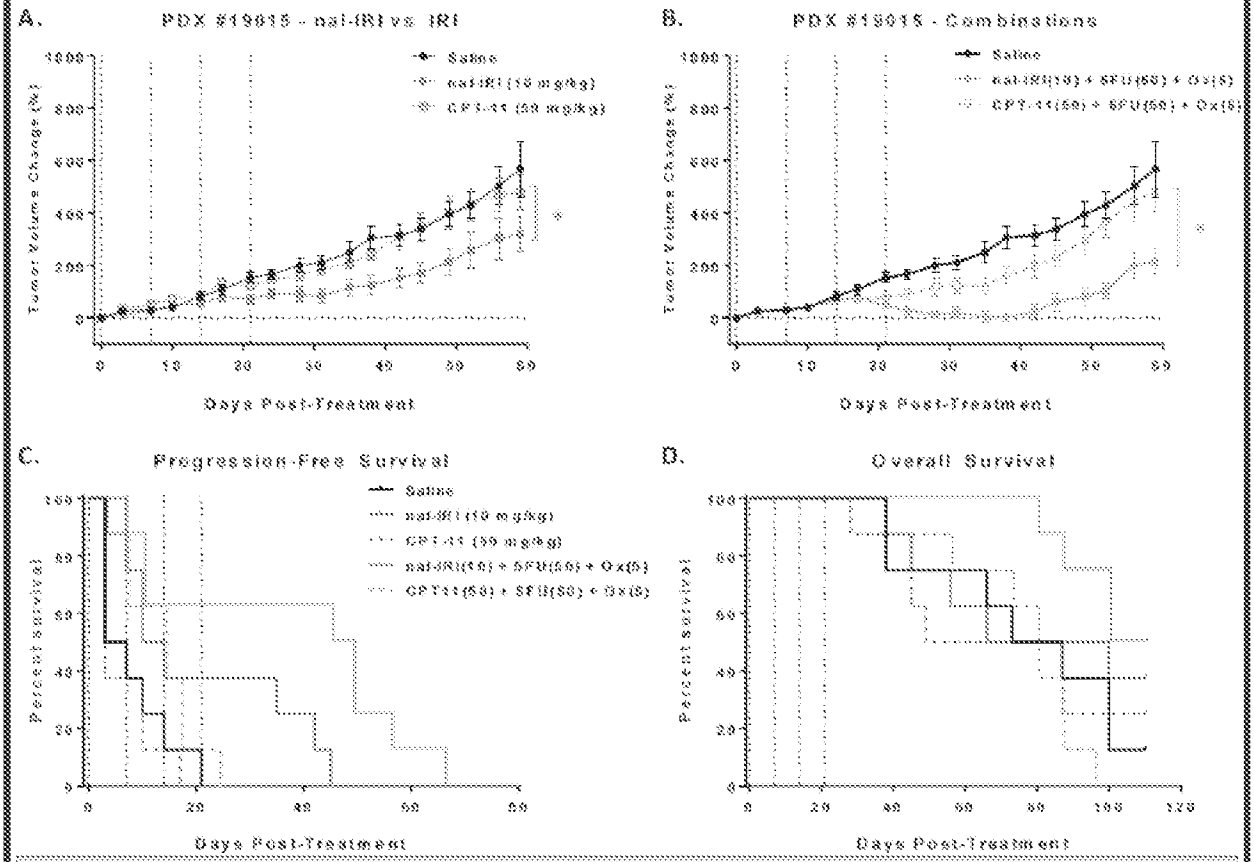


Figure 3. The combination of 5-FU and oxaliplatin with nal-IRI improves response in a patient-derived xenograft model.

A) Nal-IRI significantly improved tumor growth control relative to CPT-11. Mice received 8 weekly doses of each agent (vertical dotted lines).

B) The addition of 5-FU and oxaliplatin to both CPT-11 and nal-IRI significantly improved tumor growth control relative to the respective monotherapies (A). The nal-IRI combination was significantly more active than the CPT-11 combination.

C) Treatment with nal-IRI significantly extended PFS (median 12 days) relative to control (median 5 days) and CPT-11 (median 3 days). The nal-IRI combination with 5-FU and oxaliplatin significantly extended PFS (median 47 days) relative to nal-IRI monotherapy and the combination with CPT-11 (median 14 days). Progression: ≥30% increase in tumor volume relative to baseline.

D) The combination of nal-IRI with 5-FU and oxaliplatin significantly extended OS (median 105 days) relative to control (median 80 days) and the combination with CPT-11 (median 80 days).

NAL-IRI IMPROVES LONG-TERM TOLERABILITY OF THE COMBINATION IN MICE

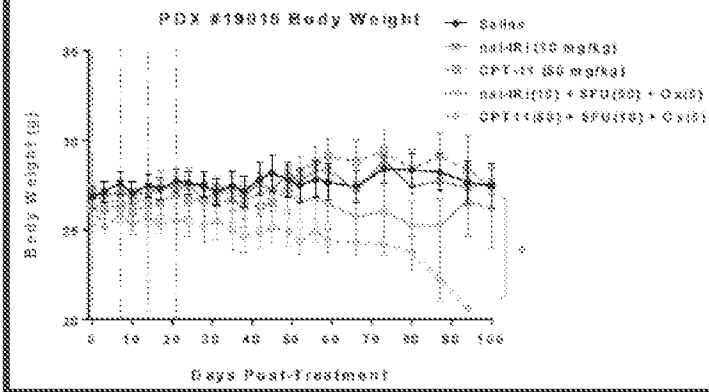


Figure 4. Nal-IRI improves tolerability following repeated dosing in mice relative to unencapsulated irinotecan when combined with 5-FU and oxaliplatin. Significance determined by ordinary 2 way ANOVA.

STAGGERED OXALIPLATIN ADMINSTRATION IMPROVES ACUTE TOXICITY IN MICE

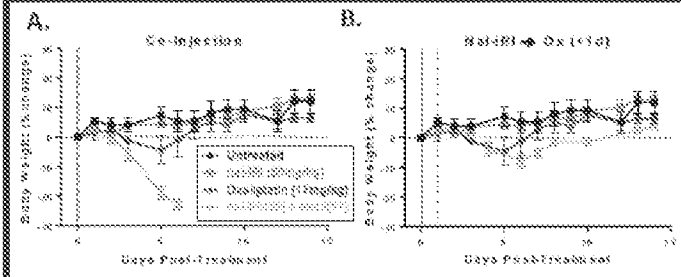


Figure 5. Acute toxicity to high doses of nal-IRI + oxaliplatin can be reduced in mice by modifying the dosing schedule of oxaliplatin.

A) Co-injection of high-dose nal-IRI and oxaliplatin resulted in rapid weight loss.

B) Oxaliplatin administration 1 day post nal-IRI treatment resulted in significantly less weight loss, comparable to oxaliplatin monotherapy.

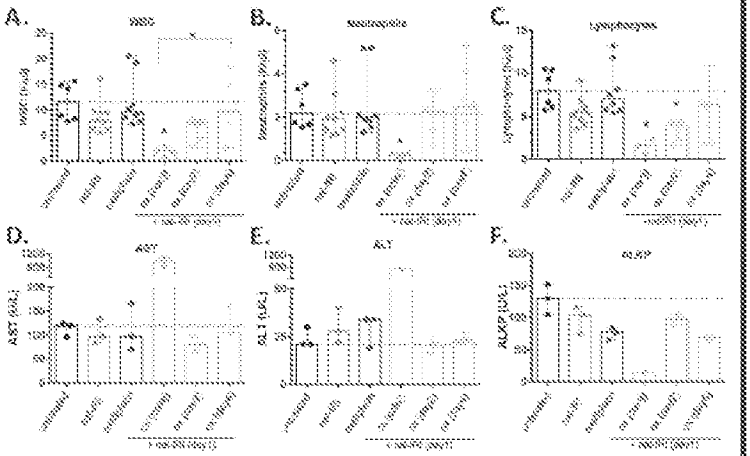


Figure 6. Delaying administration of high-dose oxaliplatin, when combined with high-dose nal-IRI, reduces hematologic and liver toxicities in mice.

- Hematologic toxicities (A-C) were improved by delayed administration of oxaliplatin.
- Liver enzymes (D-F) remained comparable to monotherapies when oxaliplatin administration was delayed.

SUMMARY

- Prolonged exposure of low-dose SN-38 simulates nal-IRI *in vitro* and increases cell growth inhibition, with and without 5-FU and oxaliplatin, in pancreatic cancer cell lines.
- Nal-IRI significantly improves tumor growth control relative to unencapsulated irinotecan/CPT-11 in *in vivo* models of pancreatic cancer.
- The combination of nal-IRI with 5-FU and oxaliplatin significantly improves tumor growth inhibition and survival relative to the combination with CPT-11.
- CPT-11 more strongly exacerbates the baseline chronic toxicities of oxaliplatin in mice than does nal-IRI.
- Delaying the administration of oxaliplatin relative to nal-IRI improves tolerability of the combination at high doses.
- Based on these preclinical data, clinical investigation is warranted and has been initiated (NCT02551991).

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Preclinical Anti-tumor Activity of Nanoliposomal Irinotecan (nal-IRI, MM-398) + 5-FU + Oxaliplatin in Pancreatic Cancer

Abstract #4830

Daniel F. Gaddy[1], Helen Lee[1], Nancy Paz[1], Shannon C. Leonard[1], Ashish Kalra[1], Jaeyeon Kim[2], Rikita A. Straubinger[2], Charlene M. Thomas[1], Robert M. Straubinger[2], Bryan M. Gillard[1], Michael T. Ahrens[1], Daryl C. Drummond[1], Stephen G. Allen[1], Dan S. Hendricks[1], Jonathan B. Fitzgerald[1]

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Preclinical Anti-tumor Activity of Nanoliposomal Irinotecan (Nal-IRI, MM-398) + 5-FU + Oxaliplatin in Pancreatic Cancer

Daniel F. Gaddy[1], Helen Lee[1], Nancy Paz[1], Shannon C. Leonard[1], Ashish Kalra[1], Ninfa L. Straubinger[2], Robert M. Straubinger[2], Bryan M. Gillard[3], Michael T. Moser[3], Daryl C. Drummond[1], Stephan G. Klinz[1], Bart S. Hendriks[1], Jonathan B. Fitzgerald[1]

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Nanoliposomal irinotecan (Nal-IRI, MM-398) recently gained approval in combination with 5-fluorouracil/leucovorin (5-FU/LV) in post-gemcitabine metastatic pancreatic cancer based on results of the Phase 3 NAPOLI-1 trial. Nal-IRI, in combination with 5-FU/LV, improved overall survival in gemcitabine-refractory metastatic PDAC relative to 5-FU/LV alone with a well-defined and manageable toxicity profile in pretreated patients. FOLFIRINOX (5-FU/LV, irinotecan and oxaliplatin) is a chemotherapy regimen active in first-line metastatic PDAC. Herein, we evaluate the preclinical anti-tumor activity of a nal-IRI + 5-FU + oxaliplatin regimen relative to the FOLFIRINOX regimen. Using pancreatic cancer cell lines, we demonstrate enhanced cell death when nal-IRI treatment is simulated using prolonged exposure of SN-38 (the active metabolite of irinotecan) in combination with 5-FU and oxaliplatin. In cell line-derived and patient-derived xenograft models of pancreatic cancer we demonstrate improved anti-tumor activity of nal-IRI relative to exposure-matched doses of unencapsulated irinotecan. Further, nal-IRI consistently improved tumor growth inhibition and survival relative to unencapsulated irinotecan in preclinical models, both as a monotherapy and in combination with 5-FU and oxaliplatin. The addition of nal-IRI to 5-FU and/or oxaliplatin did not exacerbate the baseline toxicities of these agents, including weight loss and neutropenia, and tolerability could be further improved by delaying the administration of oxaliplatin to 1 day post-MM-398. These findings illustrate the therapeutic potential of nal-IRI in combination with 5-FU/LV and oxaliplatin and support an ongoing Phase 2 trial (NCT02551991) of this triplet regimen in first-line PDAC.

Cancer Chemother Pharmacol (2012) 70:699–705
DOI 10.1007/s00280-012-1960-5

ORIGINAL ARTICLE

Phase I and pharmacokinetic study of IHL-305 (PEGylated liposomal irinotecan) in patients with advanced solid tumors

Jeffrey R. Infante · Vicki L. Keedy · Suzanne F. Jones · William C. Zamboni · Emily Chan · Johanna C. Bendell · Wooin Lee · Huali Wu · Satoshi Ikeda · Hiroshi Kodaira · Mace L. Rothenberg · Howard A. Burris III

Received: 25 June 2012 / Accepted: 16 August 2012 / Published online: 2 September 2012

Abstract
Purpose IHL-305 is a novel PEGylated liposome containing irinotecan. This study examined the safety profile and pharmacokinetics of IHL-305 and established the maximum tolerated dose and recommended phase II dose (RP2D).
Patients and methods In a standard 3 + 3 design, IHL-305 was administered IV on day 1 of a 28-day treatment schedule. Subsequently, a 14-day treatment schedule was also explored. Two patient populations were evaluated separately: Patients with at least one wild-type (*wt*) allele of UGT1A1 (UDP glucoronosyltransferase 1A1) *wt/wt* or *wt*/*28 as one group (referred to as UGT1A1 *wt* group) and patients with UGT1A1*28 homozygous variant (*28/*28) as another group.
Results Sixty patients were treated: 42 on the 28-day schedule and 18 on the 14-day schedule. Seven patients were homozygous variant (*28/*28). In the UGT1A1 *wt* group, the MTD and RP2D of IHL-305 was 160 mg/m^2 every 28 days and 80 mg/m^2 every 14 days. DLTs included nausea, vomiting, diarrhea, and neutropenia. The most common adverse events were nausea (75 %), vomiting (52 %), diarrhea (62 %), anorexia (57 %), and fatigue (57 %). At the MTD for both schedules, IHL-305 administration resulted in a high and prolonged exposure of sum total irinotecan, released irinotecan, and SN-38 in plasma. One partial response was observed in a patient with breast cancer and eight patients had stable disease for >6 months.
Conclusions IHL-305, a novel preparation of irinotecan encapsulated in liposomes, can be safely given to patients in a repeated fashion on a 4- or 2-week dosing schedule.

Keywords PEGylated liposomal irinotecan · IHL-305 · Phase I · Pharmacokinetic

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Introduction

Irinotecan is a DNA topoisomerase I inhibitor that is FDA approved for the treatment of colon cancer [1]. In order to be clinically effective, irinotecan must be converted to its active metabolite SN-38, which is then converted via UGT1A1 conjugation to its inactive metabolite SN-38 glucuronide (SN-38G) [2, 3]. Biotransformation of SN-38 to SN-38G is protective against gastrointestinal toxicity following irinotecan administration. Previous studies of every 3-week irinotecan demonstrated that patients with the homozygous UGT1A1*28 variant have a higher risk for severe neutropenia due to reduced conversion of SN-38 to SN-38G [4, 5].

IHL-305, a PEGylated liposome containing irinotecan, was developed to achieve improved bioavailability and

antitumor activity. Liposome preparations are selectively transported to tumor tissues due to the effect of enhanced permeability and retention (EPR) and their blood retention time is prolonged [6].

This first-in-human study determined the dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) of IHL-305 in UGT1A1 *wt* patients. Other objectives included evaluation of the pharmacokinetics, antitumor activity, and the potential impact of UGT1A1 genotype on the incidence and severity of adverse events.

Patients and methods

All patients provided written informed consent. The study was approved by the independent ethics committee for each site, and was conducted in accordance with the Declaration of Helsinki. The study was registered with the clinical trials registry (NCT00364143).

Patient selection

Eligibility included the following: histologically confirmed solid tumor with no known regimen of higher efficacy available; Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; normal organ and marrow function; no chemotherapy within 4 weeks; no prior irinotecan; no known brain metastases. Patients with significant cardiac disease, a history of serious ventricular arrhythmias, or a left ventricular ejection fraction (LVEF) ≤40 % were excluded.

Study design and treatments

The protocol was designed to determine the DLT and MTD of IHL-305 administered every 28 days in either homozygous wild type (*wt/wt*) or heterozygous variant (*wt/*28*) UGT1A1*28 genotype patients (referred to as UGT1A1 *wt* group). Homozygous variant patients were enrolled at reduced dosages and were not included in the MTD determination. Once the MTD for the 28-day schedule was established, the protocol was amended to change the dosing interval to 14 days and the MTD was re-determined.

A 3 + 3 dose escalation scheme was utilized with dose doubling until ≥1 patient experienced a grade 2 toxicity during cycle 1; thereafter, doses were increased in 33 % increments. UGT1A1 genotype testing was performed prior to enrollment. At least three evaluable non-*28/*28 genotype patients were treated at each dose level. Homozygote variant patients were treated at 50 % of the current dose with the option to escalate to 75 and 100 % if ≤grade 1 toxicity occurred. The recommended phase II dose was defined as the highest dose level at which ≤1 out of 6 UGT1A1 *wt* group patients experienced DLT.

The starting dose was 7 mg/m^2, which corresponded to 1/6 of the highest non-severely toxic dose in dogs (2 mg/kg). IHL-305 was administered as a 60-min infusion. Initially, no antiemetic premedications were given, but the protocol was amended to require premedications at doses >67 mg/m^2.

Assessments

UGT1A1 genotyping, electrocardiogram assessments, and laboratory assessments were performed at baseline. Physical exams were performed on day 1 of every cycle. Laboratory assessments were repeated weekly. Electrocardiograms were obtained prior to the start of infusion, at the infusion midpoint, immediately after and 2 h after the infusion during all cycles. Disease assessments were repeated every 8 weeks. Response Evaluation Criteria in Solid Tumors (RECIST 1.0) criteria were used to assess response.

Safety and tolerability

Toxicity was graded according to National Cancer Institute Common Toxicity Criteria, version 3.0 and acute DLT was determined during the first 28 days. Dose-limiting toxicity was defined as: grade 4 hematologic toxicity lasting ≥5 days; grade 3 or 4 febrile neutropenia; grade 4 thrombocytopenia; ≥grade 3 non-hematologic toxicities; prolonged QTc >500 ms; or any toxicity resulting in a treatment delay >1 week. If a patient experienced a DLT, the patient was treated at the next lower dose in subsequent cycles.

Pharmacokinetic study design and analytical studies

Heparinized blood samples were collected at the following timepoints: predose, end of infusion, 0.5, 1, 2, 4, 8, 12, and 24 h after the infusion. Once a DLT was observed, additional samples were collected 48, 72, 96, and 192 h after the infusion. Each sample was centrifuged at 3,000×*g* for 15 min at 4 °C. The plasma samples were divided into two aliquots for analysis of released irinotecan, SN-38, SN-38G, APC, and NPC, and for analysis of total irinotecan. The irinotecan total (lactone + hydroxyl acid) form of sum total (encapsulated + released) irinotecan, released irinotecan, SN-38, SN-38G, APC, and NPC concentrations were measured by high-performance liquid chromatography (HPLC) with fluorescence detection.

In the 28-day cohort, urine specimens were collected at the following timepoints: predose, 0–4, 4–8, 8–12, and 12–24 h after the start of infusion. Once a DLT was

observed, samples were also collected during the 24–48, 48–72, and 72–96-h interval after the start of infusion. The total volume of urine collected was recorded and a 1-ml sample was processed and frozen for analysis using the above HPLC assay.

Pharmacokinetic analyses

The pharmacokinetic analyses were performed using non-compartmental methods. The area under the plasma concentration versus time curve (AUC) was calculated for sum total irinotecan, released irinotecan, SN-38, SN-38G, and APC. The percent irinotecan released was calculated as [(irinotecan released AUC/irinotecan sum total AUC) × 100].

Results

Patient characteristics and disposition

Between January 2007 and November 2009, 62 patients were enrolled. Two patients never received treatment and are not included in the analysis. Sixty patients were treated: 42 on the 28-day and 18 on the 14-day schedule. Table 1 describes the patient characteristics for the UGT1A1 *wt* group patients enrolled on both schedules as well as the homozygous variant patients.

Escalation, DLT, and MTD

Thirty-six UGT1A1 *wt* group patients were enrolled across 10 dose levels ranging from 7 to 210 mg/m^2 every 28 days, Table 2. No DLTs were encountered until the 67 mg/m^2 dose level. At this dose level, one patient experienced grade 3 nausea and vomiting, and the dose level was expanded to six with no additional DLTs. All subsequent patients received prophylactic antiemetics. At 160 mg/m^2, one patient experienced grade 3 diarrhea resulting in expansion to six patients, but no additional DLTs were reported. Dose-limiting toxicities consisting of febrile neutropenia and grade 3 nausea/vomiting were reported in two patients enrolled at 210 mg/m^2, respectively. The MTD was exceeded and the RP2D was declared to be 160 mg/m^2 every 28 days.

The starting dose for the 14-day schedule was 80 mg/m^2, Table 2. One of the three patients at the initial dose experienced a 2-week treatment delay due to prolonged neutropenia, which by definition was a DLT. As a result, three additional patients were enrolled with no additional DLTs. The dose was escalated to 105 mg/m^2 and no DLTs were reported. Five patients were enrolled at the highest dose, as two were inevaluable for escalation due to rapidly progressing disease. Dose-limiting toxicities consisting of grade 3 fatigue, nausea, vomiting, and diarrhea in one patient and grade 4 neutropenia in combination with significant grade 2 diarrhea were reported in two of the three evaluable patients at 140 mg/m^2 which exceeded the MTD. The previous dose level of 105 mg/m^2 was expanded to six and DLTs (grade 3 nausea, fatigue, and anorexia as well as grade 2 fatigue warranting a 2-week treatment delay) were encountered in two patients, which exceeded the MTD. As a result, the initial dose of 80 mg/m^2 was the RP2D for the 14-day schedule.

Seven UGT1A1 **28/*28* patients were enrolled at various dose levels throughout the study. No DLTs were reported in this group. Four of the six patients treated on the 28-day schedule were removed from study at the end of cycle 1 due to disease progression (three patients) or patient request (one patient). The other two patients, who initiated treatment at 7 and 80 mg/m^2, were subsequently able to escalate IHL-305 and remained on study for six cycles each. The single UGT1A1 **28/*28* patient on the 14-day schedule received four cycles at 40 mg/m^2, but was unable to dose escalate due to grade 2 toxicities. Overall, the toxicity profile did not appear different among the UGT1A1 **28/*28* patients compared with the UGT1A1 *wt* group.

Safety and tolerability

The total number of 28-day cycles administered was 142: median two cycles/patient (range, 1–12). Nine patients (21 %) received ≥6 cycles, six (14 %) required dose reductions, and eight (19 %) required dose delays of 1–2 weeks. The total number of 14-day cycles administered was 102: median three cycles/patient (range, 1–25). Three patients (17 %) received ≥12 cycles, five (28 %) required dose reductions, and eight (44 %), including the one homozygous variant patient, required dose delays of 1–3 weeks. One patient on the 28-day schedule was switched to the 14-day schedule after 12 cycles and received an additional 10 cycles.

Table 3 describes the treatment-related toxicities for all patients, as well as divided by genotype and dosing schedule. Gastrointestinal toxicities were reported most commonly, were predominantly mild to moderate in intensity, and occurred in a slightly higher percentage of patients on the 14-day schedule. These toxicities were dose limiting in five patients. Other dose-limiting non-hematologic toxicities included fatigue and anorexia. Hematologic toxicities occurred in less than one-third of patients, but febrile neutropenia and neutropenia warranting dose reductions were dose limiting in three patients. One patient on the 28-day schedule experienced a grade 2 hypersensitivity reaction during the initial infusion, but was able to continue treatment following antihistamine and steroid administration. The patient was premedicated with steroids, H1 and

Table 1 Demographic characteristics

Demographic characteristic	UGT1A1 *wt* group (*wt/wt* and *wt/*28*) Every 28 days $N = 36$	UGT1A1 *wt* group (*wt/wt* and *wt/*28*) Every 14 days $N = 17$	Homozygous UGT1A1*28 variant (*28/*28) Both schedules $N = 7$
Age (years)			
Median	60 (41–75)	52 (35–79)	56 (42–65)
<50	8	7	1
50–69	20	7	6
70+	8	3	0
Gender			
Female	25	12	5
Male	11	5	2
Ethnic origin			
White	31	15	4
Black	4	2	3
Hispanic	1	0	0
ECOG performance status			
0	23	12	3
1	12	5	3
2	1	0	1
Prior chemotherapy			
None	1	0	1
1–3 regimens	12	13	3
≥4 regimens	23	4	3
Prior radiation therapy	17	7	7
Tumor type			
Ovary	8	1	0
Breast	7	3	1
Lung (NSCLC/SCLC)	5/0	1/1	1/1
Unknown primary	0	4	0
Pancreatic	0	3	0
Neuroendocrine	3	0	1
Bladder	2	0	0
Colorectal	2	1	0
Prostate	2	0	0
Head and neck	2	1	1
Other	5	2	2
UGT1A1 genotype			
Homozygous wild type	25	10	0
Heterozygous wild type	12	7	0
Homozygous variant	0	0	7

H2 antagonists for all subsequent cycles with no further incidents.

Efficacy

Sixty patients were treated. One partial response was reported in a metastatic breast cancer patient previously treated with five prior chemotherapy regimens. The patient received 20 cycles on the 14-day regimen and remained on study for 9.5 months. Twenty-four patients (40 %) experienced stable disease as their best response. Twenty-nine patients (48 %) experienced progressive disease at the first disease assessment. Six patients were invaluable for response.

Table 2 Dose escalation schemas (28- and 14-day dosing) for UGT1A1*28 *wt* group

Level	IHL-305 dose (mg/m^2)	Number of patients	Number of cycles[a]	Dose-limiting toxicities (cycle 1 only)
Part I every 28-day dosing				
1	7	3	6	None
2	14	3	11	None
3	28	3	5	None
4	37	3	9	None
5	50	3	9	None
6	67	6	29	1 (grade 3 nausea/vomiting)
7	88	3	18	None
8	120	4	17	None
9	160	6	34	1 (grade 3 diarrhea)
10	210	2	2	2 (febrile neutropenia; grade 3 nausea/vomiting)
Part II every 14-day dosing				
1	80	6	56	1 (Gr 2 neutropenia >2 weeks causing dose delay)
2	105	6	43	2 (Gr 3 anorexia, fatigue, nausea; >2 week treatment delay due to grade 2 fatigue)
3	140	5	8	2 (Gr 3 nausea, vomiting diarrhea; grade 4 neutropenia requiring dose reduction)

[a] Total includes cycles that were administered to patients requiring dose reductions. Three patients in part II received a total of four cycles at a reduced dose of 60 mg/m^2 and one patient received a single cycle at a dose of 52.5 mg/m^2. One patient in part I received two cycles at 90 mg/m^2

Table 3 Treatment-related toxicities for all patients by genotype and schedule ($N = 60$)

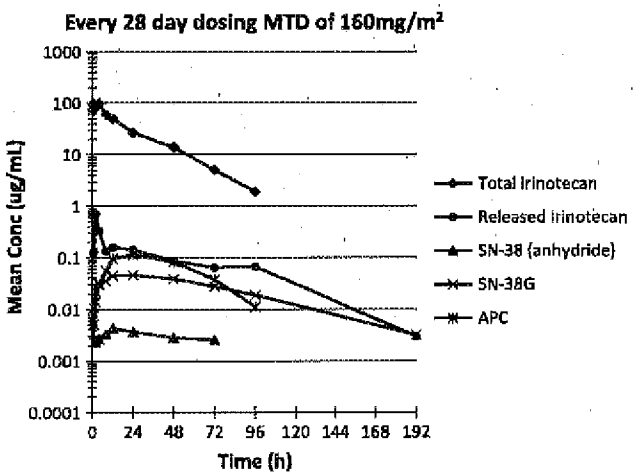
Toxicity	UGT1A1 *wt* group ($N = 36$) (*wt/wt* and *wt/*28*) Every 28-day dosing		UGT1A1 *wt* group ($N = 17$) (*wt/wt* and *wt/*28*) Every 14-day dosing		Homozygous UGT1A1*28 variant (*28/*28) ($N = 7$) Both dosing schedules		All patients ($N = 60$)
	Grade 1–2 *n* (%)	Grade 3–4 *n* (%)	Grade 1–2 *n* (%)	Grade 3–4 *n* (%)	Grade 1–2 *n* (%)	Grade 3–4 *n* (%)	Overall *n* (%)
Hematologic toxicities							
Neutropenia	0	2 (6 %)	2 (12 %)	3 (18 %)	0	1(14 %)	8 (13 %)
Febrile neutropenia	0	1 (3 %)	0	0	0	0	1 (2 %)
Anemia	5 (14 %)	4 (11 %)	7 (41 %)	0	3 (43 %)	0	19 (32 %)
Thrombocytopenia	0	0	0	0	0	0	0
Non-hematologic toxicities							
Nausea	21 (58 %)	6 (17 %)	12 (71 %)	2 (12 %)	4 (57 %)	0	45 (75 %)
Diarrhea	20 (56 %)	1 (3 %)	12 (71 %)	1 (6 %)	3 (43 %)	0	37 (62 %)
Vomiting	11 (31 %)	5 (14 %)	8 (47 %)	2(12 %)	5 (72 %)	0	31 (52 %)
Constipation	8 (22 %)	0	3 (18 %)	0	2 (29 %)	0	13 (22 %)
Fatigue	20 (56 %)	1 (3 %)	9 (53 %)	2 (12 %)	2 (29 %)	0	34 (57 %)
Peripheral edema	10 (28 %)	1 (3 %)	1 (6 %)	0	0	0	12 (20 %)
Anorexia	13 (36 %)	8 (22 %)	7 (41 %)	2 (12 %)	3 (43 %)	1 (14 %)	34 (57 %)
Dehydration	3 (8 %)	1 (3 %)	4 (24 %)	0	0	0	8 (13 %)
Alopecia	5 (14 %)	–	3 (18 %)	–	1 (14 %)	–	9 (15 %)

Pharmacokinetics

The mean concentration versus time plot for sum total irinotecan, released irinotecan, SN-38, SN-38G, and APC for the RP2D of each schedule is presented in Fig. 1. There was prolonged exposure of sum total irinotecan, released irinotecan, SN-38, SN-38G, and APC in plasma from 0 to 96 h in both schedules. The sum total form of irinotecan,

which primarily represents the encapsulated form, was 1,000–10,000-fold higher in plasma compared with released irinotecan and other metabolites.

The AUC for sum total irinotecan, released irinotecan, SN-38, SN-38G, and APC for both schedules is presented in Table 4. The inter-patient variability in the pharmacokinetics of sum total and released irinotecan was approximately two- to threefold. The mean percent of irinotecan measured in the plasma as related to sum total irinotecan ranged from 0.35 to 0.95 %. The conversion of released irinotecan to SN-38 and APC and the conversion of SN-38 to SN-38G were similar to after administration of non-liposomal irinotecan [7]. The plasma exposures of sum total and released irinotecan are relatively dose proportional; however, the small number of patients at each dose level and the inter-patient variability make this hard to evaluate.



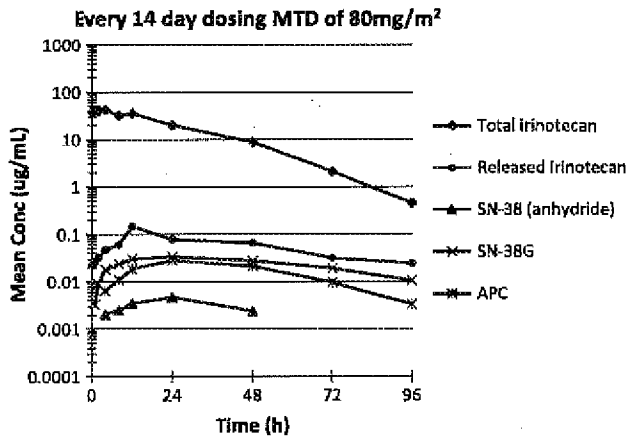


Fig. 1 Mean concentration versus time plot total irinotecan, released irinotecan, SN-38, and SN-38G at MTD for 28-day dosing (160 mg/m^2) and 14-day dosing (80 mg/m^2). NPC concentrations in plasma were below the lower limit of detection

Table 4 PK parameters for IHL-305 administered every 28 and 14 days

	Every 28-day dosing					Every 14-day dosing		
	67 mg/m^2 (0–192 h) $N = 3$	88 mg/m^2 (0–192 h) $N = 2$	120 mg/m^2 (0–192 h) $N = 4$	160 mg/m^2 (0–192 h) $N = 6$	210 mg/m^2 (0–192 h) $N = 2$	80 mg/m^2 (0–96 h) $N = 6$	105 mg/m^2 (0–96 h) $N = 6$	140 mg/m^2 (0–96 h) $N = 5$
Total irinotecan AUC_{0-t}(min–max) (h μg/ml) [mean ± SD, range]	1,250 ± 333 (974–1,620)	1,680 (1,640–1,710)	1,910 ± 824 (1,030–2,970)	2,180 ± 859 (1,370–2,870)	3,570 (1,790–5,340)	1,300 ± 364 (989–1,850)	1,810 ± 603 (1,170–2,570)	2,250 ± 1,016 (1,500–4,020)
Released irinotecan AUC_{0-t} (min–max) (h ng/ml) [mean ± SD, range]	4,380 ± 1,363 (2,910–4,640)	4,460 (2,780–6,130)	13,500 ± 12,865 (4,250–32,100)	13,800 ± 10,457 (5,930–34,700)	7,370 (7,320–7,410)	5,660 ± 3,374 (2,690–11,900)	12,500 ± 16,948 (3,910–46,600)	17,100 ± 14,979 (8,240–42,500)
SN-38 AUC_{0-t}(min–max) (h ng/ml) [mean ± SD, range]	203 ± 98 (117–310)	151 (151)	212 ± 276 (48.8–531)	245 ± 223 (46.0–661)	261 (261)	228 ± 187 (72.3–544)	312 ± 134 (237–529)	478 ± 306 (129–897)
SN-38G AUC_{0-t}(min–max) (h ng/ml) [mean ± SD, range]	2,920 ± 2,484 (1,410–5,790)	1,160 (949–1,370)	3,560 ± 2,092 (1,700–5,640)	4,100 ± 1,690 (1,720–5,910)	2,680 (1,300–4,050)	2,270 ± 1,918 (1,170–6,020)	2,960 ± 3,263 (726–9,470)	6,770 ± 7,878 (3,510–20,700)
APC AUC_{0-t}(min–max) (h ng/ml) [mean ± SD, range]	1,660 ± 719 (1,240–2,490)	1,190 (361–2,020)	2,380 ± 1,015 (1,480–3,670)	6,390 ± 7,804 (1,810–22,000)	1,090 (711–1,470)	1,490 ± 1,802 (522–4,510)	2,730–4,940 (311–12,800)	6,650 ± 5,657 (353–15,800)
Irinotecan released (%) [mean ± SD]	0.35 ± 0.11	0.26 ± 0.13	1.6 ± 0.21	0.67 ± 0.44	0.27 ± 0.2	0.45 ± 0.27	0.95 ± 1.50	0.72 ± 0.42

NPC concentrations in plasma were below the lower limit of detection

The total percent of all forms excreted in the urine over 96 h following IHL-305 administration ranged from 7.6 to 22 %.

Discussion

This first-in-human study evaluated IHL-305, a novel PEGylated liposome containing irinotecan, in patients with advanced solid tumors. In the UGT1A1 *wt* group, the MTD and RP2D of IHL-305 was 160 mg/m^2 every 28 days. When administered every 14 days, the MTD and RP2D is 80 mg/m^2.

Gastrointestinal treatment emergent adverse events were the dominant non-hematologic toxicities, occurring in 83 % of patients, and were grade 3/4 in 12 % of patients irrespective of dose and schedule. Gastrointestinal events also accounted for three of the four DLTs on the 28-day regimen, and one DLT on the 14-day regimen. Though difficult to distinguish the relationship to treatment in advanced cancer patients, fatigue, and anorexia were also observed commonly, sometimes at grade 3–4 levels. As expected with a topoisomerase-1 inhibitor, neutropenia accounted for the remaining dose-limiting toxicities. Interestingly, neutropenia only occurred in 13 % of all patients. Thrombocytopenia was not observed. These data suggest that the liposomal formulation results in predominant GI toxicities and less myelosuppression as compared with non-liposomal irinotecan [1]. Patients with the homozygous UGT1A1*28 variant had similar tolerability of IHL-305, though they were initially treated at half the dose of the UGT1A1 *wt* group. The most frequently reported adverse events remained gastrointestinal disorders (83 %) and no grade ≥3 adverse events were reported.

The patient population included patients with multiple prior therapies from a variety of tumor types. Among the 54 patients with restaging scans, there was one confirmed partial response despite all patients being irinotecan naive. This patient was treated at 140 mg/m^2 in the 14-day cohort (later reduced to 105 mg/m^2). Eight patients (13 %) had stable disease >6 months.

Administration of IHL-305 results in a high and prolonged exposure of sum total irinotecan, released irinotecan, SN-38, and other metabolites. In addition, the release of irinotecan from IHL-305 in plasma is low (<1 %). These results are consistent with other nanoparticle and liposomal formulations of camptothecin analogues and other anticancer agents [8–11]. The inter-patient variability in the sum total irinotecan was significantly lower after IHL-305 (two- to threefold) compared with administration of other PEGylated liposomal agents and nanoparticle agents [8–11]. The factors associated with the lower inter-patient variability in the IHL-305 pharmacokinetics are unknown. The conversion of released irinotecan to SN-38 is similar to the conversion following non-liposomal irinotecan. Thus, administration of IHL-305 does not appear to alter the pharmacokinetics of irinotecan and metabolites once the drug is released from the liposome. However, the exposure of sum total and released irinotecan and SN-38 after IHL-305 is significantly prolonged compared with non-liposomal irinotecan [7]. The prolonged exposure of irinotecan and SN-38 is expected to lead to an increased exposure of SN-38 in tumor. Theoretically, the delivery of encapsulated and released irinotecan to the tumor may result in intra-tumoral conversion of released irinotecan to SN-38; however, proving this in patients has been difficult.

IHL-305 can be safely given to patients in a repeated fashion using an every 4- or 2-week schedule. The liposome formulation resulted in a high and prolonged exposure of both sum total irinotecan and the active metabolite SN-38. Gastrointestinal side effects and neutropenia defined the MTD, but were manageable. The limited responses may be due to the heavily pretreated patient population and the majority of patients being treated at doses below the RP2D. Randomized trials are needed to evaluate if IHL-305 offers clinical benefit compared with non-liposomal irinotecan.

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Cancer Research

Preclinical activity of nanoliposomal irinotecan is governed by tumor deposition and intratumor pro-drug conversion

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Title: Preclinical activity of nanoliposomal irinotecan is governed by tumor deposition and intratumor pro-drug conversion

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***Notes:** AV Kalra and J Kim contributed equally to the manuscript

Abstract

A major challenge in the clinical use of cytotoxic chemotherapeutics is maximizing efficacy in tumors while sparing normal tissue. Irinotecan is used for colorectal cancer treatment but the extent of its use is limited by toxic side-effects. Liposomal delivery systems offer tools to modify pharmacokinetic and safety profiles of cytotoxic drugs. In this study, we defined parameters that maximize the antitumor activity of a nanoliposomal formulation of irinotecan (nal-IRI). In a mouse xenograft model of human colon carcinoma, nal-IRI dosing could achieve higher intratumoral levels of the pro-drug irinotecan and its active metabolite SN-38 compared to free irinotecan. For example, nal-IRI administered at doses 5-fold lower than free irinotecan achieved similar intratumoral exposure of SN-38 but with superior antitumor activity. Tumor response and pharmacokinetic modeling identified the duration for which concentrations of SN-38 persisted above a critical intratumoral threshold of 120 nM as determinant for antitumor activity. We identified tumor permeability and carboxylesterase activity needed for pro-drug activation as critical factors in achieving longer duration of SN-38 in tumors. Simulations varying tumor permeability and carboxylesterase activity predicted a concave increase in tumor SN-38 duration, which was confirmed experimentally in 13 tumor xenograft models. Tumors where higher SN-38 duration was achieved displayed more robust growth inhibition compared to tumors with lower SN-38 duration, confirming the importance of this factor in drug response. Overall, our work shows how liposomal encapsulation of irinotecan can safely improve its antitumor activity in preclinical models by enhancing accumulation of its active metabolite within the tumor microenvironment.

Introduction

Liposomal carriers have become clinically accepted in cancer therapy as delivery systems that can enhance the utility of existing anticancer drugs (1). The potential benefits of these macromolecular carriers include overcoming solubility issues for certain drug classes, protecting the drug from unwanted metabolism and extending the residence time in plasma and tissue. In particular, liposomes tend to preferentially accumulate in tumors as a result of an enhanced permeability and retention (EPR) effect. The EPR effect is attributed to the abnormal tumor vasculature permitting extravasation of macromolecules, as well as impaired lymphatic drainage that promote the retention of these molecules within the tumor microenvironment, thereby providing sustained release at the tumor site mimicking a metronomic dosing (2). Increased tumor deposition via the EPR effect may also prevent drug resistance by overcoming the activity of multidrug resistant proteins (3,4) and may offer possible means of improving safety aspects by reducing systemic exposure relative to tumor exposure (5). There are potential pharmacologic advantages of the EPR effect, particularly for antineoplastic agents that have to engage their target over a longer time period or have little binding activity; an example is drugs of the camptothecin class with topoisomerase 1 enzyme (TOP1) as the primary target.

Irinotecan (CPT-11), a clinically approved camptothecin, is a pro-drug that is activated by CES enzymes, present primarily in liver and colon tissue to the active form, SN-38. The active SN-38 can be subsequently inactivated through glucuronidation by members of the UDPglucuronosyltransferase family (6). The principal mechanism of action leading to cell death is through DNA damage after replication-fork collisions with transient drug-TOP1 cleavage complexes, thus emphasizing the time of drug exposure as important driver for cytotoxicity of camptothecins (7,8). Recently, we described the development of a novel nanoliposomal formulation of irinotecan, nal-IRI (also known as MM-398 or PEP02) (9). nal-IRI features very high drug loading efficiency, a high drug payload, and marked *in vivo* drug retention that also stabilizes the active lactone configuration of irinotecan. The pharmacokinetic properties of the encapsulated irinotecan were dramatically altered in the plasma of female rats, with a

344x increase in the area under the curve (AUC), an 8.5x decrease in the volume of distribution and a 39.6x increase in the half-life of the total drug. Pharmacokinetic analysis in a clinical study confirmed these performance characteristics of nal-IRI in patients (10).

Plasma drug concentrations cannot readily be translated into therapeutic effect; a sufficient amount of active therapeutic agent must be transported to the tumor site of action (i.e. be available for uptake by cancer cells) in order to observe favorable drug activity (11). The transport of macro-molecules across the tumor vasculature is a complex process depending on vessel perfusion, surface area, and permeability, as well as tumor and drug characteristics. Several studies have utilized mathematical models to understand liposomal drug delivery within solid tumors (12,13). Of particular interest is work done by Hendriks et al., where the authors constructed a computational model to describe the parameters that affect the tumor delivery of pegylated liposomal doxorubicin, the first liposomal anticancer agent to receive clinical approval (14). The study concluded that liposome PK and tumor permeability to liposomes (tumor deposition) were the most important parameters controlling liposomal drug delivery to tumors.

In the case of nal-IRI, the complex metabolism (15) and mechanism of action (8,16) of free irinotecan, in addition to the above mentioned parameters, may play a role in the overall liposomal irinotecan delivery within tumors. In the present study, we describe a systems pharmacology approach to identify critical parameters that differentiate nal-IRI from free irinotecan with regard to *in vivo* activity. A mechanistic tumor PK model was developed and trained to describe CPT-11 and SN-38 levels observed in plasma and tumor, following administration of either nal-IRI or free irinotecan in tumor xenografts. A model sensitivity analysis was performed to identify the critical parameters driving *in vivo* activity, which were then experimentally confirmed by measuring these factors in multiple cell-line and patient-derived xenograft models. The findings in the present study highlight critical parameters that could serve as potential biomarkers to identify cancer indications and patient populations with an increased likelihood of nal-IRI responsiveness.

Material & Methods

Materials and nal-IRI preparation

nal-IRI was prepared as previously described (9) using a lipid composition of DSPC, Cholesterol, and PEG-DSPE (3:2:0.015, mol: mol:mol), an initial drug-to-lipid ratio of 500 g drug/mol phospholipid, and extrusion through 0.1 um polycarbonate filters. The resulting preparations displayed a particle size of 111 nm (with polydispersity index of 0.04), and a drug load of 473 mg irinotecan-HCl/mmol phospholipid. All lipids were obtained from Avanti Polar Lipids Inc. Irinotecan hydrochloride was purchased from the pharmacy. Acetic acid, methanol and acetonitrile were from EMD Chemicals Inc. Water and trifluoroacetic acid (TFA) were from J. T. Baker. Fetal Bovine Serum was from Tissue Culture Biologicals and phosphate buffered saline (PBS) was purchased from Life Technologies.

Cell culture

Cell lines (HT-29 (colon), SK-ES-1 (Ewing's sarcoma), A549 (lung), LoVo (colon), MDA-MB-231 (breast)) were obtained from ATCC whereas A2780 cells (ovarian) was obtained from Sigma-Aldrich. Cells from ATCC and Sigma were received in 2010. All cells were authenticated prior to receipt and were propagated for less than six months after resuscitation. Cultures are regularly tested for mycoplasma. All cell lines were cultured in humidified CO_2 atmosphere at $37^{O}C$ using media recommended by the manufacturer.

Pharmacokinetic and tissue bio-distribution study

Five-week-old female NOD-SCID mice were purchased from Charles River Laboratory. The care and treatment of experimental animals were in accordance with Institutional Animal Care and Use Committee guidelines. Subcutaneous tumors were established by injecting 10 million HT-29 cells into the right flank of mice. When the average tumor volume reached approximately 200 mm^3, mice were randomized into groups (n=4/time point) that received a single intravenous (i.v.) dose of nal-IRI at 5

mg/kg, 10 mg/kg, 20 mg/kg or 40 mg/kg. Following 1, 4, 8, 24, 48, 72 and 168 hours after a single dose, mice were sacrificed and perfused with PBS prior to harvest tumor and other normal tissues.

Antitumor activity studies

Five-week-old female NOD-SCID mice were purchased from Charles River Laboratory . Subcutaneous tumors were established by injecting 10 million HT-29 and SK-ES-1 cells or 5 million A549 cells into the right flank of mice. Tumor growth was measured twice per week by calipers and calculated with formula: width^2×length×0.52. When the average tumor volume reached approximately 200 mm^3, mice were randomized into treatment groups (n=5-8/group) that received weekly i.v. dose of PBS (control), free irinotecan (50 mg/kg) or nal-IRI at various doses ranging from 1.25 to 20 mg/kg.

Tumor growth inhibition (TGI) was calculated with formula:

$$\mathrm{TGI}(\%) = \left[1 - \frac{(V_{treated}(d_{final}) - V_{treated}(d_0))}{(V_{control}(d_{final}) - V_{control}(d_0))}\right] \qquad \text{(A)}$$

where, $V_{treated}$ and $V_{control}$ represent the volumes of tumor at a given time point following treatment with drug or PBS ; d_0 and d_{final} represent first day and final day of treatment, respectively.

Characterizing tumors from cell-line and patient-derived xenografts

The cell-line derived xenografts (HT-29, SK-ES-1, A549, MDA-MB-231, LoVo, AsPC-1, A2780) were established as described above. The patient-derived tumor models [CTG-0062 (colorectal), CTG-0079 (colorectal), CTG-0252 (ovarian), CTG-0288 (pancreatic), CTG-0158(lung), CTG-0283 (pancreatic)] were established by Champions Oncology using their Champions TumorGraft® (CTG) technology. When the average tumor volume reached approximately 300 mm^3, mice were randomized into treatment groups (n=4/group) that received single i.v. dose of either PBS or nal-IRI at 10 mg/kg. Prior to tumor collection, intracardial perfusion was performed to remove the blood components from the tumor compartment. Briefly, a butterfly needle (23G) connected to a 10 ml syringe filled with PBS is inserted into the left ventricle. Inferior vena cava is cut and animal is perfused with 10 ml PBS (within 1-2 mins). The control tumors were harvested 24 hours after PBS administration and used for the irinotecan activation assay,

whereas the treated tumors were harvested either 24 hours or 72 hours following nal-IRI treatment and used for HPLC analysis.

HPLC quantification of CPT-11 and SN-38

Tumor and normal tissues were analyzed for CPT-11 and SN-38 concentrations using a modification of the method previously described (9). Briefly, tissues were weighed and homogenized for 2 minutes in 20% w/v water using a TissueLyser (Qiagen). The homogenates were extracted by mixing 0.1 ml homogenate with 0.9 ml 1% acetic acid/methanol followed by 10 s vortexing and placing at −80°C for 1 hour. The samples were centrifuged at 10,000 rpm for 10 minutes at room temperature and supernatants collected for HPLC analysis (Dionex). The samples and standards (CPT-11 and SN-38) were analyzed using a C18 reverse phase column (Synergi Polar-RP 80A 250×4.60 mm 4 μm column). The drug metabolites were eluted running a gradient from 30% acetonitrile; 70% 0.1% TFA/H_2O to 68% acetonitrile; 32% 0.1% TFA/H_2O during a 13 minutes span at a flow rate of 1.0ml/min. The initial elute composition was restored after 14 minutes and continued for 6 minutes before the next injection. The CPT-11 peak was detected at ~7.7 minutes and the SN-38 peak eluted at ~8.4 minutes, using an in-line fluorescence detector excited at 372 nm and emitting at 556 nm.

Irinotecan activation assay

Tumor tissue lysates were prepared by homogenizing the tissue in 6% w/v 0.1M Tris HCL/1% Triton X-100 solution (pH7.5) using a TissueLyser for 2-4 minutes. Protein concentration of lysates was measured using the BCA reagent (Thermo Scientific). Lysates (250 µg of protein) were mixed with an equal volume of 10 µM irinotecan and incubated at 37°C. Following 24 hours of incubation the reaction was terminated by adding an equal volume of 1% acetic acid/methanol and samples centrifuged at 10,000 rpm for 15 minutes. The supernatant was processed for HPLC quantification of CPT-11 and SN-38 as described above.

Statistical analysis

The statistical significance of differences between groups was analyzed with one way ANOVA test. Results were considered statistically significant at $p<0.05$. The analysis was performed using GraphPad Prism 6.01

Model development and simulation

Pharmacokinetic profiles of metabolites in plasma and tumor from free irinotecan and nal-IRI were described by using multi-compartmental models (Fig. 1B). The model equations are explained and summarized in the supplementary materials. The models were built and implemented using Simbiology® toolbox in MATLAB® 8.2 (The MathWorks®).

Results

nal-IRI displays a prolonged exposure in both plasma and tumor compared to free irinotecan

The pharmacokinetic profiles of the pro-drug CPT-11 and its active metabolite SN-38 were measured in plasma and tumors following administration of either free irinotecan or nal-IRI (Fig. 1A). At similar doses of both free irinotecan and nal-IRI, the CPT-11 and SN-38 plasma levels cleared rapidly from circulation within 8 hours after free irinotecan injection, whereas the levels of CPT-11 and SN-38 following nal-IRI administration were persistent and remained in circulation for over 50 hours. A ~10 fold higher plasma

CPT-11 peak level was observed with nal-IRI as compared to free irinotecan. However, the plasma peak level of SN-38 achieved with nal-IRI was 10 fold lower compared to free irinotecan, probably due to the ability of the lipid bilayer to protect the conversion of pro-drug CPT-11 to SN-38 by the systemic CES enzyme present in mouse models (17). Administration of free irinotecan resulted in the clearance of greater than 90 percent of CPT-11 from tumors within 24 hours; however, following nal-IRI administration, CPT-11 levels persisted above 10,000 nM levels for 168 hours. Similar peak levels of SN-38 were achieved with both free irinotecan and nal-IRI in HT-29 tumors, though a prolonged SN-38 exposure for up to 168 hours (measured as the AUC from 0 to 168 hours) was achieved with nal-IRI as compared to less than 48 hours tumor exposure with free irinotecan. In summary, CPT-11 and SN-38 were still present in tumors at 168 hours following nal-IRI administration, though both CPT-11 and SN-38 had cleared from plasma.

Tumor SN-38 duration drives *in vivo* activity

We developed a mechanistic PK model to identify the determinants that may differentiate the plasma and tumor PK profiles between free irinotecan and nal-IRI (Fig. 1B). The experimental PK data were used to estimate the optimal model parameters (Table 1) fitting the model simulations within the standard deviations of *in vivo* PK profiles of both CPT-11 and SN-38 (Fig. 1A). As the *in vitro* cytotoxic effects of irinotecan on tumor cells is dependent on the concentration and the time of exposure of cells to active metabolite SN-38 (7,8), we sought to understand if the overall plasma and tumor SN-38 exposure predicts the *in vivo* activity of both nal-IRI and free irinotecan. The trained model determined that a five-fold higher dose of free irinotecan (50 mg/kg) was required to achieve similar SN-38 exposure in both plasma and tumor as compared to nal-IRI (10 mg/kg) (Fig. 2A). The tumor growth inhibition of HT-29 xenograft model at these equal exposure doses, was significantly greater with nal-IRI (~110%) treatment as compared to free irinotecan (~40%), despite the five-fold lower total dose administered (*$p<0.05$, one way ANOVA test) (Fig. 2B). In addition, other studies have shown no additional HT-29 tumor growth inhibition at the maximum tolerated dose of free irinotecan (100 mg/kg) (18). In order to identify a dose level of nal-IRI that gave comparable in vivo activity to 50 mg/kg free irinotecan, we performed a dose

escalation study in the HT-29 xenograft model (Fig. 2C). nal-IRI at 5 mg/kg showed partial inhibition of tumor growth (~40% TGI) that was comparable to 50 mg/kg free irinotecan, whereas 10 mg/kg and 20 mg/kg nal-IRI showed significant (*$p<0.05$, one way ANOVA test) tumor growth inhibition compared to saline (~110-130% TGI). Furthermore, we have previously tested control liposomes (that have comparable composition to nal-IRI except for the absence of irinotecan, the active pharmaceutical ingredient) and did not observe any tumor growth inhibition (data not shown).

The intratumor SN-38 concentrations achieved from 50 mg/kg and 100 mg/kg doses of free irinotecan and 1.25, 2.5, 5, 10, and 20 mg/kg doses of nal-IRI were then simulated using the trained mechanistic PK model (Fig. 2D). Although a nal-IRI dose of 5 mg/kg achieved similar TGI as 50 mg/kg free irinotecan, the tumor SN-38 AUC and peak levels were ~2-fold and ~6-fold lower respectively for nal-IRI as compared to free irinotecan. Further, we noted at these doses both drugs were able to maintain the tumor SN-38 concentration above 120 nM for the same duration of ~40 hours. In order to determine if the tumor SN-38 concentration impacts *in vivo* activity, we used the tumor SN-38 concentration of 120 nM as a threshold. We also determined the duration for which the various doses of nal-IRI or free irinotecan could maintain the tumor SN-38 concentration above 120 nM, hereon referred to as "tumor SN-38 duration". A sigmoidal relationship between TGI (%) and tumor SN-38 duration (Fig 2E) was observed for both nal-IRI and free irinotecan (R^2=0.62). However, when comparing TGI (%) to tumor SN-38 AUC (Fig. 2F) the relationship was less significant (R^2=0.45), due to the lower TGI (%) achieved by 50 mg/kg free irinotecan compared to 10 mg/kg nal-IRI. We also observed longer SN-38 duration in tumors (> 100 hours) compared to normal tissues (< 72 hours). (Fig. 2G and Supplementary Fig. S1).

Identification of liposome tumor permeability and local tumor activation as critical determinants for tumor SN-38 duration

A local sensitivity analysis on the model parameters was performed to identify processes impacting the tumor SN-38 duration (Supplementary materials). In response to the administration of free irinotecan (50 mg/kg), the tumor SN-38 duration was relatively insensitive to most model parameters (Fig. 3A),

suggesting the inability of free irinotecan to modulate it. In contrast, several model parameters were found to significantly impact tumor SN-38 duration following the administration of nal-IRI (10 mg/kg) (Fig. 3B). The sensitive parameters for nal-IRI can be classified into three different categories: (i) PK, rate of breakdown of liposomes in blood (Release rate in blood, $V_{max,Release,p}$), (ii) activation of pro-drug CPT-11 to SN-38 by CES enzyme (CES activity in tumor; $V_{max,CES,t}$ and blood; $V_{max,CES,p}$), and (iii) liposome uptake within tumors i.e nal-IRI tumor deposition (nal-IRI tumor permeability, $PS_{nal\text{-}IRI}$). Among these parameters, the release rate in plasma negatively affected tumor SN-38 duration due to a decrease in the overall systemic exposure of nal-IRI. CES enzyme activity, particularly from tumor CES (local tumor activation of irinotecan) and nal-IRI permeability (tumor deposition), positively affected the tumor SN-38 duration. In order to assess the identifiability of parameter estimates, log likelihood profiling was performed for the sensitive parameters, $V_{max,CES,t}$ and $PS_{nal\text{-}IRI}$ (19). The confidence intervals suggested that both parameters were precisely estimated (Supplementary Fig. S2).

Biological variability and simulated perturbation of nal-IRI tumor deposition and local activation

To determine the biological relevance of these sensitive parameters towards driving tumor SN-38 duration (namely nal-IRI tumor deposition and local tumor activation of irinotecan), the parameters were measured in a panel of 13 xenograft models. We used the total CPT-11 concentrations in tumors as a surrogate for nal-IRI tumor deposition as model simulations based on nal-IRI pharmacokinetics showed that majority of CPT-11 in plasma and tumor was encapsulated and protected within the liposomes and less than 10% was available as free CPT-11 (Supplementary Fig. S3). The intra tumor concentrations of CPT-11 varied substantially across the tumor panel (Fig. 4A). The tumor models from cell-lines displayed overall higher levels of pro-drug CPT-11 deposition (from 5000 - 15000 ng/g) as compared to patient-derived tumor models (1000 - 2000 ng/g). In addition, a high degree of variability was observed between individual tumors within the same xenograft model (66% average coefficient of variation). Model simulations were used to test the effect of altering nal-IRI tumor deposition on tumor SN-38 duration (Fig. 4B). By decreasing the nal-IRI permeability parameter to zero, which simulates an impermeable

tumor microenvironment, the tumor SN-38 duration of ~100 hours achieved with 10 mg/kg nal-IRI was substantially reduced to ~50 hours and approached the levels observed with 50 mg/kg free irinotecan. Taken together, these results suggest that the tumor deposition of nal-IRI is highly tumor specific and will dramatically impact tumor SN-38 duration.

To determine the degree to which local tumor activation of irinotecan varied in human tumors, we measured CES activity using an *ex vivo* assay in a panel of 80 patient-derived tumors. The tumor lysates varied in their ability to activate pro-drug irinotecan and produce SN-38 (1-25 ng/ml SN-38 produced), suggesting a high degree of variability in local tumor activation of irinotecan across indications. A significant difference in local tumor activation of irinotecan was observed between colon and lung tumors ($p<0.05$). However, there was no significant difference between other indications, which may be due to high variability observed within each indication (Fig. 4C). The impact of varying the tumor CES activity on tumor SN-38 duration was evaluated by simulating a knock-out of tumor CES enzyme (Fig. 4D). In the absence of local tumor activation, tumor SN-38 duration with nal-IRI (10 mg/kg) decreased from ~100 hours to ~40 hours, similar to that achieved by free irinotecan (50 mg/kg).

nal-IRI tumor deposition and local activation collectively predict tumor SN-38 duration

The relative contribution of nal-IRI tumor deposition and local tumor activation on tumor SN-38 duration was evaluated using model simulations. Based on the findings from the sensitivity analysis (Fig. 3B), nal-IRI permeability ($PS_{nal\text{-}IRI}$) and tumor CES activity ($V_{max,CES,t}$) values were utilized to create a map relating these parameters to tumor SN-38 duration following nal-IRI administration (Fig. 5A). Model simulations predicted a concave relationship, where the tumor SN-38 duration is dependent upon both the tumor permeability and the tumor CES activity. The tumor SN-38 duration could be increased by either increasing the $PS_{nal\text{-}IRI}$ or $V_{max,CES,t}$ (white arrows) and the maximum tumor SN-38 duration of 168 hours was only reached with CES activity at 0.025nmol/min and tumor permeability at 1.5E-4 L/min/kg.

To experimentally test the model predictions, we used the same panel of 13 xenograft models to measure the tumor concentrations of CPT-11 (as a surrogate for tumor deposition, supplementary Fig.

S4A); tumor SN-38 concentrations (as a surrogate for tumor SN-38 duration; supplementary Fig. S4B) and CES activity (for local tumor activation of irinotecan). The experimental data supported the model simulations, confirming that the SN-38 concentration within tumors was dependent on both the tumor CPT-11 concentration and tumor CES activity (Fig 5B). All tumor models with high CPT-11 concentration >2000 ng/g or high CES activity > 5 ng/ml displayed high tumor SN-38 concentrations ("red") ranging from 25 – 125 ng/ml (Supplementary Table 2). In certain tumor models, one of the parameters contributed predominantly towards higher SN-38 concentrations (black arrows). A2780 and SK-ES-1 tumors displayed high tumor SN-38 concentrations of 97 ng/ml and 127 ng/ml respectively (Supplementary Table 2), which was mainly due to high CPT-11 concentrations (>2000 ng/g) whereas in other tumor models (CTG-0062 and AsPC-1) the CES activity (>5 ng/ml) was the dominant factor contributing towards high tumor SN-38 concentrations. Further tumor models with the lowest tumor SN-38 concentrations ranging from 5 – 12 ng/ml ("blue"), including several patient-derived tumor models (boxed area) also displayed lower tumor CPT-11 concentrations (<2000 ng/g) and CES enzyme activity (<5 ng/ml).

Tumor SN-38 duration correlates with nal-IRI *in vivo* activity

In vivo tumor response studies were performed in three tumor models where different tumor SN-38 durations had been observed (as indicated by tumor SN-38 concentration at 72 hours) to determine the impact of tumor SN-38 duration on *in vivo* activity of nal-IRI. The tumor volumes observed for both HT-29 (Fig. 6A) and SK-ES-1 (Fig. 6B) models were significantly lower ($p<0.05$) following 10 mg/kg nal-IRI as compared to untreated tumors. In both these models, tumor regression was observed immediately after the first dose and was sustained through the course of the study. A549 tumors achieved lower SN-38 tumor levels (Fig. 4A) and did not respond to nal-IRI treatment (Fig. 6C). Interestingly, both A549 and HT-29 cells displayed similar *in vitro* sensitivity to SN-38 with IC_{50} values of 53 nM and 44 nM respectively (20). In summary, nal-IRI induced stronger responses (~100%TGI) in tumor models that had higher tumor SN-38 duration (> ~100 hours).

Discussion

The nal-IRI formulation dramatically alters the pharmacological properties of irinotecan as well as its active metabolite, SN-38 (9). In this study, we identified a pharmacological parameter - namely, tumor SN-38 duration - as a driver of irinotecan-based *in vivo* activity and propose biomarkers that can impact tumor SN-38 duration achieved by nal-IRI. Our study indicates that nal-IRI can completely inhibit tumor growth compared to free irinotecan, despite administering doses that achieve similar SN-38 exposure (measured as the AUC). Instead, the duration of prolonged exposure of SN-38 within tumors achieved by nal-IRI was shown to be a major pharmacological determinant for *in vivo* activity in mice.

Several studies have shown improved *in vitro* cytotoxic activity of SN-38 when cells are exposed to drug for longer duration (21). The *in vitro* cell doubling time for HT-29 cells is approximately 20 hours (21), whereas *in vivo* the tumor volume doubles (Fig. 2B) at a slower rate (~8 to 9 days). In addition, at a given time only 35 to 50% of cells are in the S-phase of cell cycle wherein the maximum cytotoxicity of free irinotecan has been observed (21). Thus, in order to exert maximum cytotoxic effects across different cell cycle phases, the cells have to be exposed to free irinotecan across multiple cell cycles. Our *in vivo* study confirms these findings as the free irinotecan is rapidly cleared from plasma and tumor tissue (tumor SN-38 duration of ~40 hours), thereby not allowing sufficient time for tumor cells to be exposed to SN-38 (for only 2 cell cycle doubling time) as compared to more than 5 cell cycle doubling times with nal-IRI (tumor SN-38 duration for >100 hours). Thus the extended exposure of tumor cells to SN-38, which is achieved by nal-IRI, can contribute towards the enhanced cytotoxicity as compared to free irinotecan.

We observed higher tumor concentrations of CPT-11 and SN-38 at 168 hours following administration of nal-IRI. In contrast, the peak plasma concentrations of SN-38 was lower with nal-IRI as compared to free irinotecan, suggesting that most of the CPT-11 from nal-IRI remains inside the liposomes and is protected from systemic conversion as described with free irinotecan (17). In addition, prolonged SN-38 duration from nal-IRI administration was observed only in tumors and much less in normal tissues,

suggesting that toxicity might not be exacerbated by nal-IRI treatment. The preferentially accumulation of nal-IRI in tumors as compared to normal tissues can be attributed to the enhanced permeability and retention (EPR) effect, where the leaky vasculature in tumor facilitates the extravasation of liposomal nanoparticles and the defective lymphatic drainage helps increase the retention within tumor (1,2). Thus, with the EPR effect, nal-IRI creates a large depot of CPT-11 only in tumors thereby prolonging tumor SN-38 duration. In contrast, free irinotecan can easily be transported in and out of the tissues with a short plasma half-life, resulting in minimal SN-38 duration in tumors.

The enhanced *in vivo* activity of nal-IRI as compared to free irinotecan was attributed to the ability of nal-IRI to extend the tumor SN-38 duration. Sensitivity analysis identified two key determinants that impact the ability of nal-IRI to extend tumor SN-38 duration - (i) nal-IRI tumor deposition, as measured by the extent of pro-drug CPT-11 deposition within tumors and (ii) nal-IRI local activation, from pro-drug CPT-11 to SN-38 facilitated by the local tumor CES enzyme. The experimental data, in this study supported the importance of each of these determinants. We observed high degree of variability in the overall nal-IRI tumor deposition across the 13 xenograft models that were tested. Several studies have highlighted a role for tumor permeability, tumor perfusion, and stromal matrix in limiting the delivery of therapeutic agents into tumors (22). In our model simulations, when the nal-IRI tumor permeability was decreased to zero, the benefit of higher tumor SN-38 duration with nal-IRI was negatively impacted and reduced to levels simulated for free irinotecan. We also observed that the tumors with lower nal-IRI deposition had considerable lower SN-38 tumor levels. This data is consistent with other findings suggesting that a dense tumor stroma can impede drug permeability and limit drug delivery within tumors (23,24).

Use of tumor CES activity as a cellular parameter for predicting free irinotecan response had limited success both in preclinical (25,26) and clinical studies.(27). Through the sensitivity analysis performed in this study, we identified CES activity as a critical parameter for nal-IRI activity. Tumor models that displayed high ability to activate CPT-11, achieved high tumor SN-38 concentrations despite limited

deposition of CPT-11, thus suggesting the importance of local tumor CES enzyme expression in facilitating longer SN-38 exposure following nal-IRI administration. In fact, others have shown that *in vitro* and *in vivo* activity of free irinotecan can be enhanced by overexpressing of CES enzyme in tumor cells (28,29). In addition to tumor cells expressing CES enzyme (30), other components of the intracellular matrix such as tumor-associated macrophages (TAMs) express CES1 enzyme and play a role in CPT-11 activation (31). In fact, we performed *in vitro* studies that confirmed the ability of TAMs to hydrolyze CPT-11 to SN-38 (Supplementary Fig. S5). Thus our data suggests, the extended tumor PK achieved by nal-IRI provides high local depot of pro-drug CPT-11 for prolonged time thus allowing for activation by tumor CES enzymes. Collectively our data provides rational for investigating tumor CES enzyme activity as potential marker for nal-IRI activity.

Pharmacogenetic and pharmacodynamics markers such as Topo1 have shown limited correlations with free irinotecan response (6,32–34). In addition to the intrinsic sensitivity of tumor cells to SN-38, our data indicates that the duration for which tumor cells are exposed to SN-38 (tumor SN-38 duration) also plays a critical role in driving treatment response to irinotecan. Tumor models with extended SN-38 duration (HT-29, SK-ES-1) showed robust *in vivo* response to nal-IRI, whereas A549 with shorter tumor SN-38 duration did not respond to therapy. The fact that *in vitro* sensitivity of both HT-29 and A549 to SN-38 is very similar (20) corroborates the finding that the duration of SN-38 is driving the tumor response.

In conclusion, our data demonstrate that nal-IRI enhances the pharmacokinetic profile of tumor SN-38, prolonging tumor exposure to SN-38 compared with free irinotecan, and therefore has the potential for therapeutic effect in human cancers. Liposome permeability and CES activity were the critical factors that emerged from model simulation of tumor SN-38 duration, which were experimentally shown to vary across and within tumor indications. Thus, translational research exploring the utility of tumor liposome permeability and local activation of irinotecan as biomarkers for nal-IRI clinical activity is warranted.

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Footnote

In the manuscript, CPT-11 is used when referring to the pro-drug levels in plasma or tumor samples following either free irinotecan or nal-IRI administration. SN-38 is used when referring to the active metabolite of CPT-11.

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Table 1. Summary of model parameters for plasma PK and tumor deposition models

Name	Value	Units	Description	Reference
Plasma PK Model Parameters				
For free irinotecan				
Cl_{CPT-11}	1.222e-4	L/min	Plasma clearance rate of CPT-11	Estimated
Cl_{SN-38}	2.138e-5	L/min	Plasma clearance rate of SN-38	Estimated
$k_{12,CPT-11}$	8.444e-5	1/min	Rate of CPT-11 transport from plasma to peripheral compartment	Estimated
$k_{21,CPT-11}$	4.213e-2	1/min	Rate of CPT-11 transport from peripheral to plasma compartment	Estimated
$k_{12,SN-38}$	2.656e-5	1/min	Rate of CPT-11 transport from plasma to peripheral compartment	Estimated
$k_{21,SN-38}$	3.44e-4	1/min	Rate of CPT-11 transport from peripheral to plasma compartment	Estimated
$V_{max,CES,p}$	2.263e-1	nmol/min	Maximum rate coefficient for CES enzyme in plasma compartment	Estimated
$K_{m,CES,p}$	2.67e5	nM	Michaelis-Menten constant for CES enzyme in plasma compartment	Estimated
V_p	9.46e-5	L	Volume of plasma compartment	Estimated
V_{ph}	0.02	L	Volume of peripheral compartment	Fixed (14)
For nal-IRI				
$Cl_{nal-IRI}$	1.87e-7	L/min	Plasma clearance rate of nal-IRI	Estimated
Cl_{CPT-11}	1.634e-5	L/min	Plasma clearance rate of CPT-11	Estimated
Cl_{SN-38}	2.957e-6	L/min	Plasma clearance rate of SN-38	Estimated
$k_{12,CPT-11}$	1.619e-4	1/min	Rate of CPT-11 transport from plasma to peripheral compartment	Estimated
$k_{21,CPT-11}$	5.349e-7	1/min	Rate of CPT-11 transport from peripheral to plasma compartment	Estimated
$V_{max,Release,p}$	8.443e-6	nmol/min	Maximum rate coefficient for CPT-11 release from nal-IRI in plasma compartment	Estimated
$K_{m,Release,p}$	2.04	nM	Michaelis-Menten constant for CPT-11 release from nal-IRI in plasma compartment	Estimated
$V_{max,CES,p}$	5.943e-2	nmol/min	Maximum rate coefficient for CES enzyme in plasma compartment	Estimated
$K_{m,CES,p}$	1.198e5	nM	Michaelis-Menten constant for CES enzyme in plasma compartment	Estimated
V_p	1.12e-3	L	Volume of plasma compartment	Estimated
V_{ph}	0.02	L	Volume of peripheral compartment	Fixed (14)
Tumor Deposition Model Parameters				
Q_{tumor}	2.119e-6	L/min	Blood flow rate to tumor	(14)
$PS_{nal-IRI}$	7.858e-5	L/min/kg	Tissue permeability coefficient of nal-IRI	Estimated
PS_{CPT-11}	1.851e-3	L/min/kg	Tissue permeability coefficient of CPT-11	Estimated
PS_{SN-38}	2.687e-2	L/min/kg	Tissue permeability coefficient of SN-38	Estimated
$\sigma_{nal-IRI}$	3.181e-3		Tissue-capillary partition coefficient of nal-IRI	Estimated
σ_{CPT-11}	5.24e-1		Tissue-capillary partition coefficient of CPT-11	Estimated
σ_{SN-38}	2.109e-1		Tissue-capillary partition coefficient of SN-38	Estimated
$k_{Release,t}$	1.681e-4	1/min	Rate coefficient for +CPT-11 release from nal-IRI in tumor tissue compartment	Estimated
$V_{max,CES,t}$	2.17e-2	nmol/min	Maximum rate coefficient for CES enzyme in tumor tissue compartment	Estimated
$K_{m,CES,t}$	2.3e6	nM	Michaelis-Menten constant for CES enzyme in tumor tissue compartment	Estimated
V_{cap}	7e-7	L	Volume of tumor capillary compartment	(14)
V_t	1e-5	L	Volume of tumor tissue compartment	Fixed

Figure Legends

Figure 1. Pharmacokinetic profile of nal-IRI and free irinotecan. (A) Plasma and tumor PK of nal-IRI were compared to free irinotecan in HT-29 xenograft bearing mice. NOD SCID mice bearing HT-29 tumors were treated with single intravenous dose of free irinotecan or nal-IRI. Plasma and tumors were collected at various intervals and the CPT-11 and SN-38 were measured by HPLC analysis (n=4 animals / time point). Plasma PK data for free irinotecan were taken from Kaneda et al. (35). *Solid lines*, represent the model simulations for nal-IRI, whereas *dashed lines* represent the model simulations for free irinotecan. (B) Diagram of the mechanistic tumor pharmacokinetic model developed to describe the various steps in metabolism, pharmacokinetics and tumor deposition of nal-IRI.

Figure 2. Relation of nal-IRI *in vivo* activity to tumor SN-38 duration. (A) Model predictions for similar SN-38 AUC in plasma and tumor following free irinotecan (50 mg/kg) and nal-IRI (10 mg/kg) administration. (B) Tumor response observed in HT-29 xenograft following weekly administration (*arrows*) of 50 mg/kg free irinotecan and 10 mg/kg nal-IRI (n=8/group). The tumor volumes for nal-IRI (10 mg/kg) were significantly lower (*$p<0.05$) compared to saline and irinotecan groups (one way ANOVA test) (C) Tumor response in HT-29 xenografts following weekly administration (*arrows*) of various nal-IRI doses (n=5 /group). The tumor volumes for nal-IRI (10 mg/kg) and nal-IRI (20 mg/kg) groups was significantly lower (*$p<0.05$) compared to saline tumors on Day 25 and Day 28 (one way ANOVA test). (D) Model simulations were used to compare tumor SN-38 concentration following the administration of varying doses of free irinotecan or nal-IRI. *Black dashed line* represents threshold concentration of 120 nM to determine tumor SN-38 duration. (E) TGI(%) achieved by nal-IRI and free irinotecan treatment in HT-29 xenografts were compared to the tumor SN-38 duration above 120 nM (E) and SN-38 AUC (F) at varying doses of nal-IRI or free irinotecan *Solid lines* represent non-linear regression lines based on five parameter logistic curve fitting. (G). The SN-38 duration over a threshold of 120 nM was computed from the pharmacokinetic profiles of SN-38 in tumor and normal tissues following 20 mg/kg of nal-IRI.

Figure 3. Model parameters impacting tumor SN-38 duration. Sensitive analyses for free irinotecan (A) and nal-IRI (B) were performed on key model parameters which are responsible for plasma clearance, tissue deposition and metabolic reactions. Parameters whose values were not estimated in this study including compartment volumes and tumor blood flow were excluded from the analysis. The doses of free irinotecan (50 mg/kg) and nal-IRI (10 mg/kg) that achieved similar SN-38 plasma and tumor exposure were used for sensitivity analysis. The model parameters were modulated by 10% and their effect on tumor SN-38 duration was determined as a sensitivity index (Equation S6).

Figure 4. *In vivo* variability in nal-IRI tumor deposition and local activation. (A) Intra tumor CPT-11 concentrations were measured across cell-line derived (HT-29, SK-ES-1, A549, MDA-MB-231, LoVo, AsPC-1, A2780) and patient-derived (CTG-0062, CTG-0079, CTG-0252, CTG-0288, CTG-0158, CTG-0283) tumor models. Tumor bearing mice were administered a single intravenous dose of 10 mg/kg nal-IRI and tumors excised 24 hr later. CPT-11 concentrations were determined in the tumor lysates using HPLC analysis as described in methods (n=4-8 tumors/model). (B) The effect of nal-IRI permeability on tumor SN-38 concentrations was simulated by reducing the nal-IRI permeability parameter, $PS_{nal\text{-}IRI}$ to zero. The simulated tumor SN-38 concentrations were compared at the equal exposure doses of 10 mg/kg nal-IRI and 50 mg/kg free irinotecan . Black solid line: nal-IRI (10 mg/kg) with base $PS_{nal\text{-}IRI}$. Gray solid line: nal-IRI (10 mg/kg) with zero $PS_{nal\text{-}IRI}$. Black dashed line: free irinotecan (50 mg/kg). *Dotted line* represents threshold concentration of 120 nM (C) Carboxylesterase (CES) activity for 80 patient-derived xenograft tumors across different indications was determined using *ex vivo* irinotecan activation assay. Tumor lysates (250 μg of protein) from untreated mice was incubated with free irinotecan (5 μM) for 24 hr at 37^{O}C and the amount of SN-38 produced was measured with HPLC analysis (*$p<0.05$; t-test). (D) The effect of knocking out tumor CES activity on tumor SN-38 duration was simulated by reducing the tumor CES parameter, $V_{max,CES,t}$ to zero. The simulated tumor SN-38 concentrations were compared at the equal exposure doses of 10mg/kg nal-IRI and 50 mg/kg free irinotecan. Black solid line: nal-IRI 10 mg/kg with base $V_{max,CES,t}$. Gray solid lines: nal-IRI 10 mg/kg with zero $V_{max,CES,t}$. Black dashed line: free irinotecan 50 mg/kg with base $V_{max,CES,t}$. *Dotted line* represents threshold concentration of 120 nM

Figure 5. nal-IRI tumor deposition and local activation impacts tumor SN-38 duration. (A) The effect of changing tumor CES activity and nal-IRI permeability parameters (*arrows*) on tumor SN-38 duration (*color-coded in hours*) in tumors was simulated. The optimal parameter values for HT-29 were marked with the symbol '*'. (B) Experimental data in tumor xenograft models showing the impact of tumor CPT-11 and CES activity on tumor SN-38 concentrations. Tumor CES activity (as surrogate for local tumor activation of irinotecan) and tumor CPT-11 concentration at 72 hr (as surrogate for tumor deposition) for different xenograft models were plotted and color-coded based on their SN-38 concentrations in the tumor 72 hr after nal-IRI (each data point represents median of n=4-8 tumors). *Dotted arrows* indicate dependence of tumor SN-38 concentrations on tumor CPT-11 concentration and CES activity.

Figure 6. *In vivo* tumor response for nal-IRI. NOD SCID mice were inoculated with HT-29; colon (A), SK-ES-1; ewings (B) and A549; lung (C) cell lines. Tumor bearing mice were randomized when the tumor volume was approximately 200 mm^3. Each group received weekly intravenous dose (*arrows*) of either saline (●) or 10mg/kg dose of nal-IRI (○). Tumor volumes were measured twice per week (n=5-10 animals/group).

Figure 1B

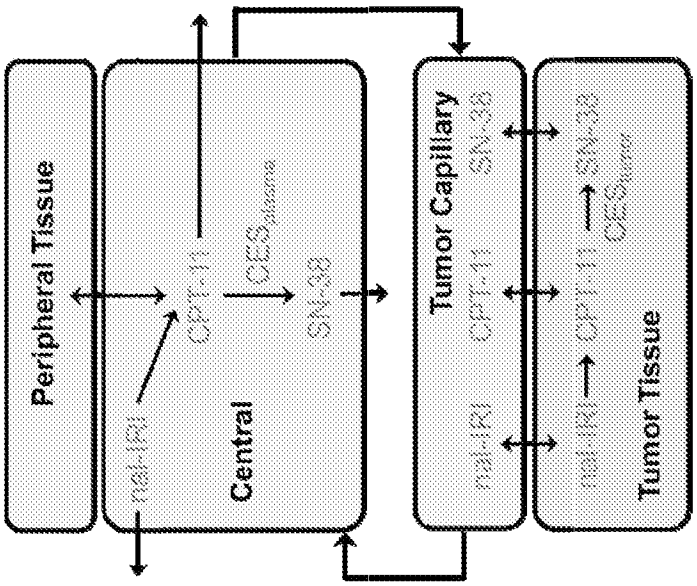
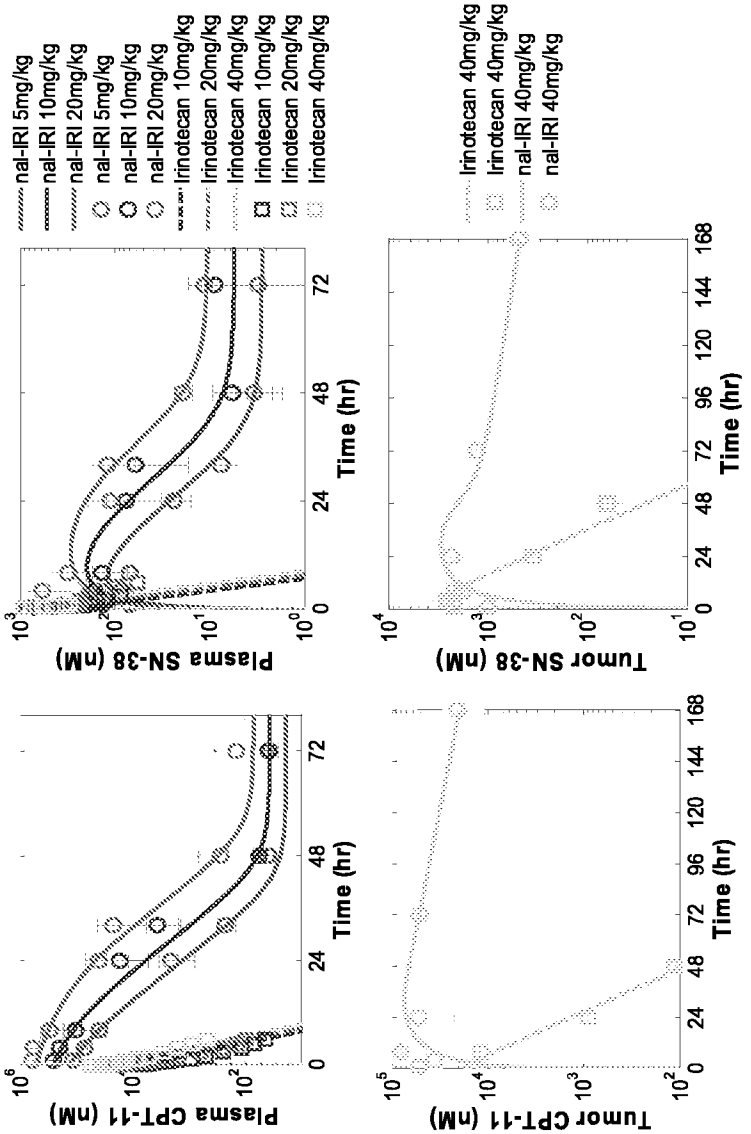


Figure 1A



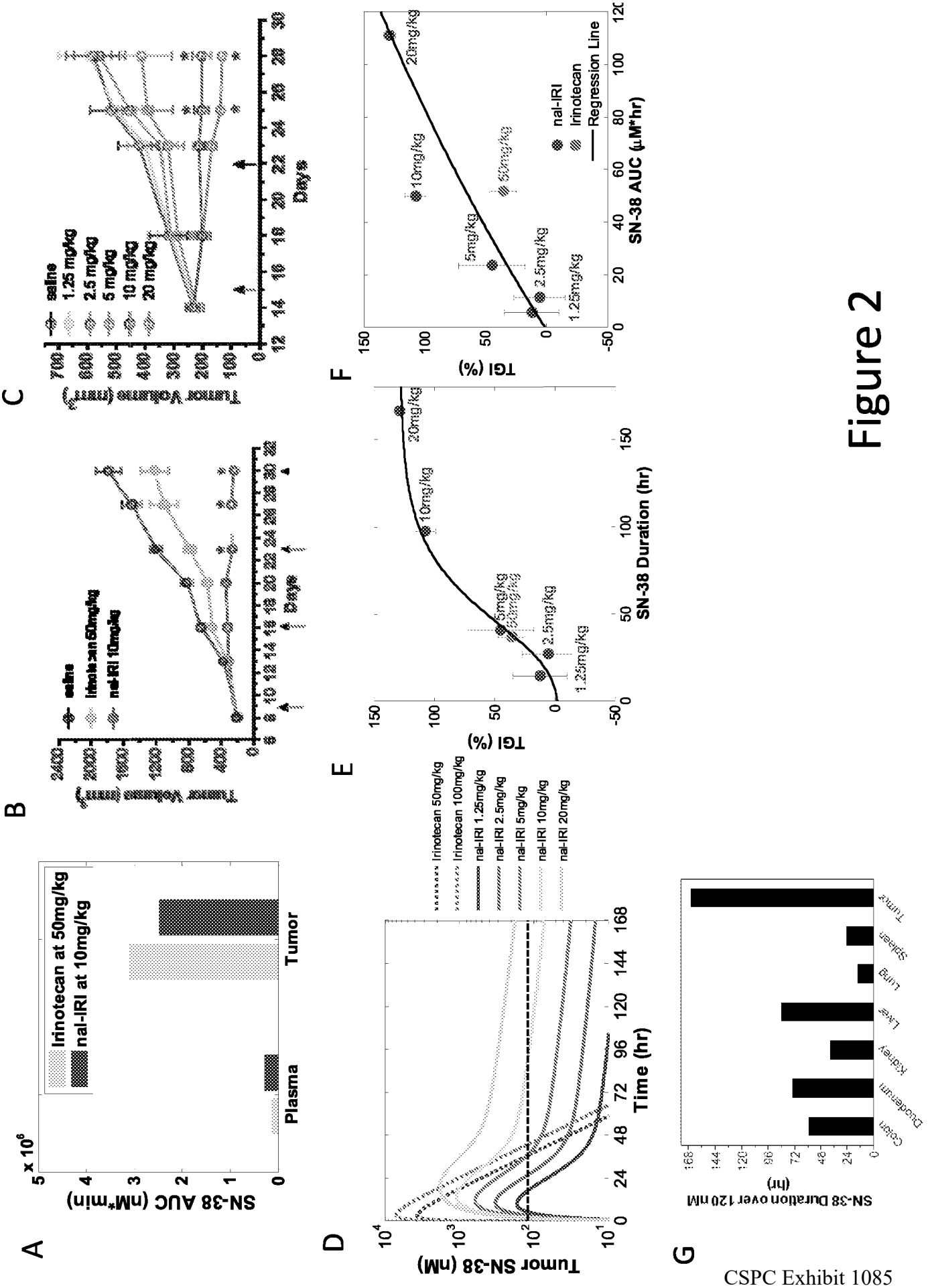


Figure 2

Figure 3

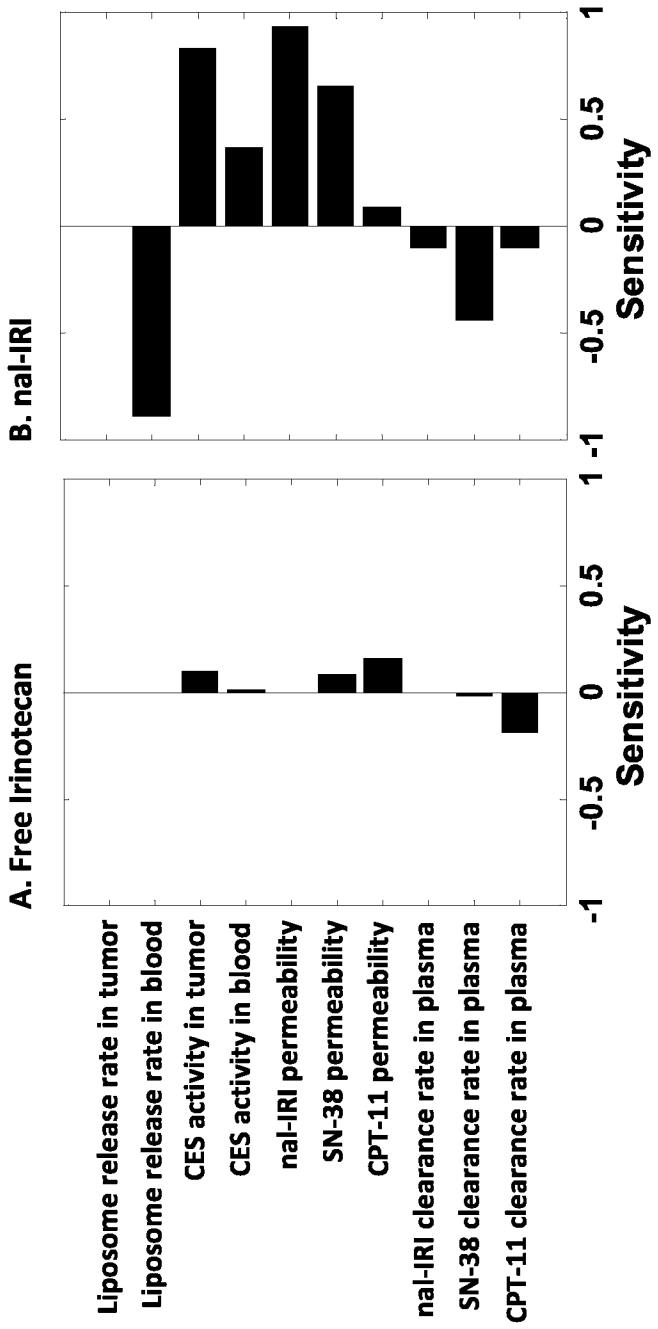
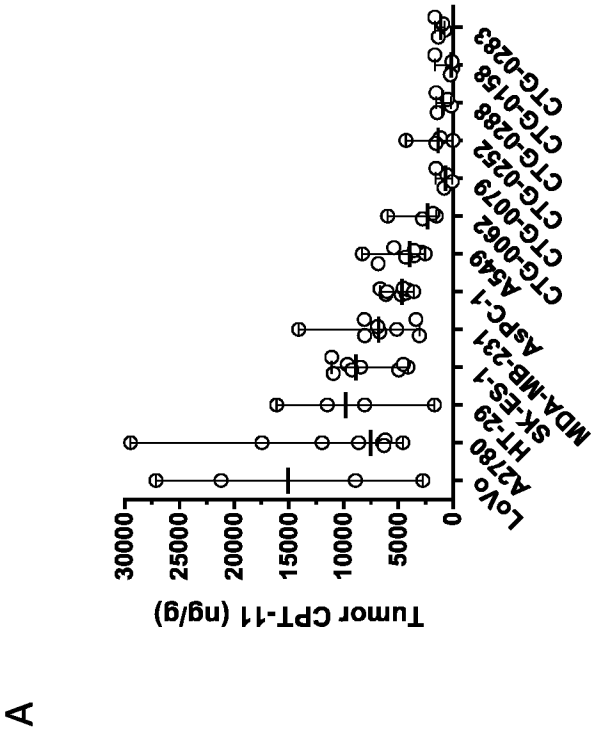
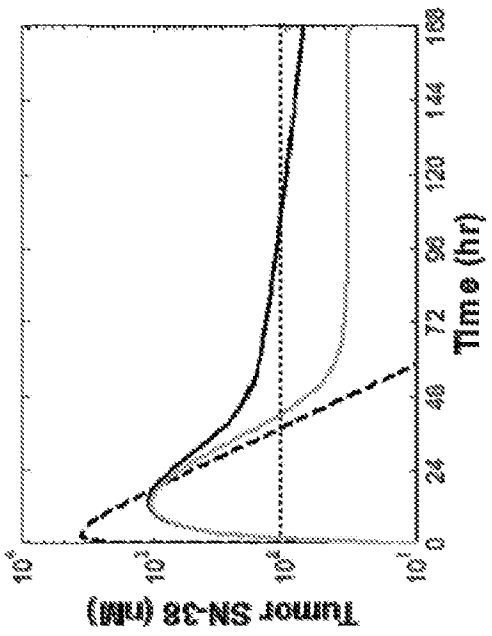
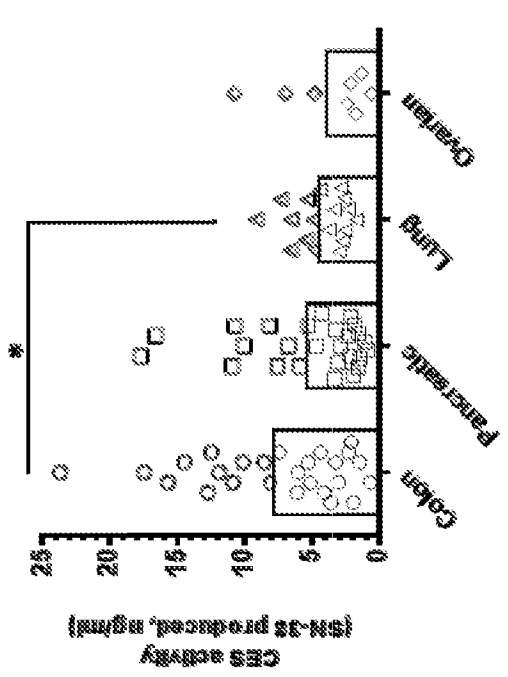


Figure 4







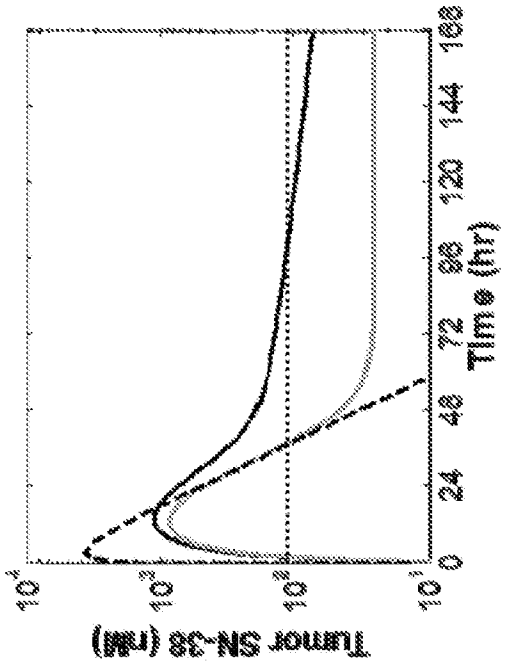


Figure 5

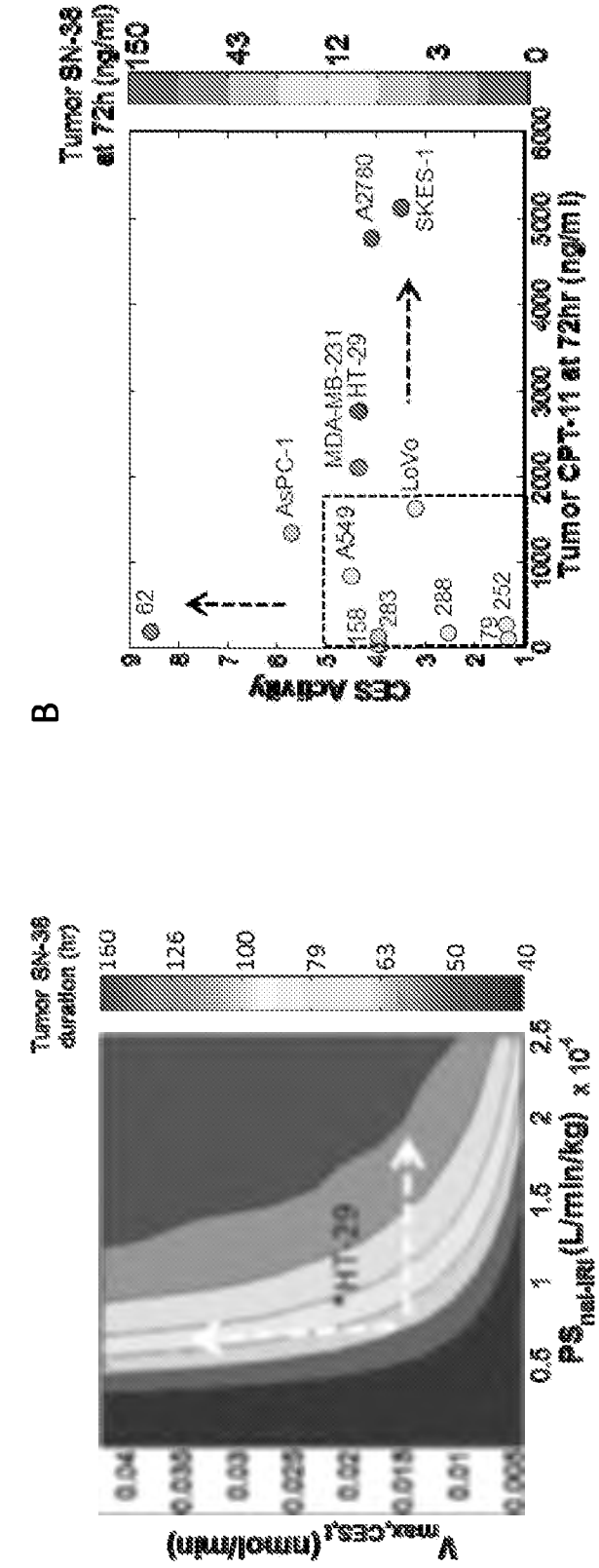
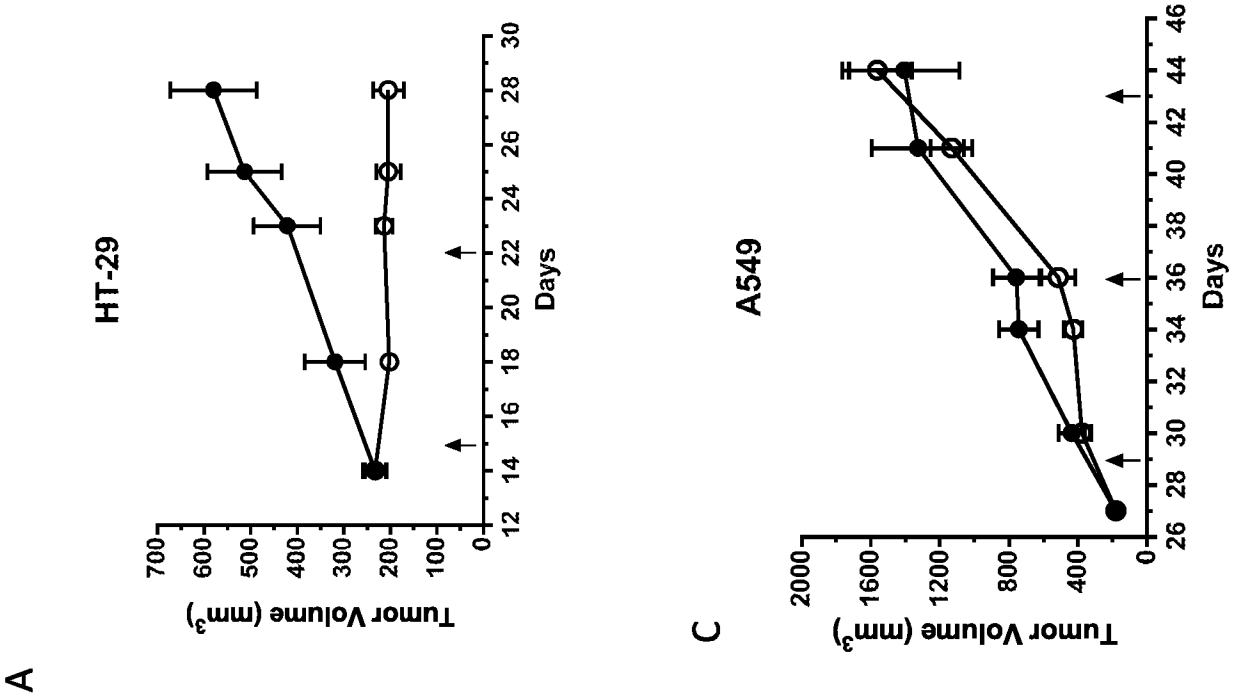


Figure 6



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Cancer Research

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Preclinical Activity of Nanoliposomal Irinotecan Is Governed by Tumor Deposition and Intratumor Prodrug Conversion

Ashish V. Kalra, Jaeyeon Kim, Stephan G. Klinz, Nancy Paz, Jason Cain, Daryl C. Drummond, Ulrik B. Nielsen, and Jonathan B. Fitzgerald

Abstract

A major challenge in the clinical use of cytotoxic chemotherapeutics is maximizing efficacy in tumors while sparing normal tissue. Irinotecan is used for colorectal cancer treatment but the extent of its use is limited by toxic side effects. Liposomal delivery systems offer tools to modify pharmacokinetic and safety profiles of cytotoxic drugs. In this study, we defined parameters that maximize the antitumor activity of a nanoliposomal formulation of irinotecan (nal-IRI). In a mouse xenograft model of human colon carcinoma, nal-IRI dosing could achieve higher intratumoral levels of the prodrug irinotecan and its active metabolite SN-38 compared with free irinotecan. For example, nal-IRI administered at doses 5-fold lower than free irinotecan achieved similar intratumoral exposure of SN-38 but with superior antitumor activity. Tumor response and pharmacokinetic modeling identified the duration for which concentrations of SN-38 persisted above a critical intratumoral threshold of 120 nmol/L as determinant for antitumor activity. We identified tumor permeability and carboxylesterase activity needed for prodrug activation as critical factors in achieving longer duration of SN-38 in tumors. Simulations varying tumor permeability and carboxylesterase activity predicted a concave increase in tumor SN-38 duration, which was confirmed experimentally in 13 tumor xenograft models. Tumors in which higher SN-38 duration was achieved displayed more robust growth inhibition compared with tumors with lower SN-38 duration, confirming the importance of this factor in drug response. Overall, our work shows how liposomal encapsulation of irinotecan can safely improve its antitumor activity in preclinical models by enhancing accumulation of its active metabolite within the tumor microenvironment. ***Cancer Res; 74(23); 1–11. ©2014 AACR.***

Introduction

Liposomal carriers have become clinically accepted in cancer therapy as delivery systems that can enhance the utility of existing anticancer drugs (1). The potential benefits of these macromolecular carriers include overcoming solubility issues for certain drug classes, protecting the drug from unwanted metabolism and extending the residence time in plasma and tissue. In particular, liposomes tend to preferentially accumulate in tumors as a result of an enhanced permeability and retention (EPR) effect. The EPR effect is attributed to the abnormal tumor vasculature permitting extravasation of macromolecules, as well as impaired lymphatic drainage that promote the retention of these molecules within the tumor microenvironment, thereby providing sustained release at the tumor site mimicking a metronomic dosing (2). Increased tumor deposition via the EPR effect may also prevent drug resistance by overcoming the activity of multidrug resistant proteins (3, 4) and may offer possible means of improving safety aspects by reducing systemic exposure relative to tumor exposure (5). There are potential pharmacologic advantages of the EPR effect, particularly for antineoplastic agents that have to engage their target over a longer time period or have little binding activity; for example drugs of the camptothecin class with topoisomerase 1 enzyme (TOP1) as the primary target.

Irinotecan (CPT-11), a clinically approved camptothecin, is a prodrug that is activated by carboxylesterase (CES) enzymes, present primarily in liver and colon tissue to the active form, SN-38. (In the article, CPT-11 is used when referring to the prodrug levels in plasma or tumor samples following either free irinotecan or nal-IRI administration. SN-38 is used when referring to the active metabolite of CPT-11.) The active SN-38 can be subsequently inactivated through glucuronidation by members of the UDP glucuronosyltransferase family (6). The principal mechanism of action leading to cell death is through DNA damage after replication-fork collisions with transient drug-TOP1 cleavage complexes, thus emphasizing the time of drug exposure as important driver for cytotoxicity of camptothecins (7, 8). Recently, we described the development of a novel nanoliposomal formulation of irinotecan, nanoliposomal formulation of irinotecan (nal-IRI; also known as MM-398 or PEP02; ref. 9). nal-IRI features very high drug loading efficiency, a high drug payload, and marked *in vivo* drug retention that

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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also stabilizes the active lactone configuration of irinotecan. The pharmacokinetic (PK) properties of the encapsulated irinotecan were dramatically altered in the plasma of female rats, with a 344× increase in the area under the curve (AUC), an 8.5× decrease in the volume of distribution, and a 39.6× increase in the half-life of the total drug. Pharmacokinetic analysis in a clinical study confirmed these performance characteristics of nal-IRI in patients (10).

Plasma drug concentrations cannot readily be translated into therapeutic effect; a sufficient amount of active therapeutic agent must be transported to the tumor site of action (i.e., be available for uptake by cancer cells) to observe favorable drug activity (11). The transport of macromolecules across the tumor vasculature is a complex process depending on vessel perfusion, surface area, and permeability, as well as tumor and drug characteristics. Several studies have used mathematical models to understand liposomal drug delivery within solid tumors (12, 13). Of particular interest is work done by Hendriks and colleagues (14), where the authors constructed a computational model to describe the parameters that affect the tumor delivery of pegylated liposomal doxorubicin, the first liposomal anticancer agent to receive clinical approval. The study concluded that liposome PK and tumor permeability to liposomes (tumor deposition) were the most important parameters controlling liposomal drug delivery to tumors.

In the case of nal-IRI, the complex metabolism (15) and mechanism of action (8, 16) of free irinotecan, in addition to the above-mentioned parameters, may play a role in the overall liposomal irinotecan delivery within tumors. In this study, we describe a systems pharmacology approach to identify critical parameters that differentiate nal-IRI from free irinotecan with regard to ***in vivo*** activity. A mechanistic tumor PK model was developed and trained to describe CPT-11 and SN-38 levels observed in plasma and tumor, following administration of either nal-IRI or free irinotecan in tumor xenografts. A model sensitivity analysis was performed to identify the critical parameters driving ***in vivo*** activity, which were then experimentally confirmed by measuring these factors in multiple cell line and patient-derived xenograft models. The findings in this study highlight critical parameters that could serve as potential biomarkers to identify cancer indications and patient populations with an increased likelihood of nal-IRI responsiveness.

Materials and Methods

Materials and nal-IRI preparation

nal-IRI was prepared as previously described (9) using a lipid composition of DSPC, cholesterol, and PEG-DSPE (3:2:0.015, mol:mol:mol), an initial drug-to-lipid ratio of 500 g drug/mol phospholipid, and extrusion through 0.1 μm polycarbonate filters. The resulting preparations displayed a particle size of 111 nm (with polydispersity index of 0.04), and a drug load of 473 mg irinotecan-HCl/mmol phospholipid. All lipids were obtained from Avanti Polar Lipids Inc. Irinotecan hydrochloride was purchased from the pharmacy. Acetic acid, methanol, and acetonitrile were from EMD Chemicals Inc. Water and trifluoroacetic acid (TFA) were from J.T. Baker. Fetal bovine serum was from Tissue Culture Biologicals and phosphate-buffered saline (PBS) was purchased from Life Technologies.

Cell culture

Cell lines [HT-29 (colon), SK-ES-1 (Ewing's sarcoma), A549 (lung), LoVo (colon), MDA-MB-231 (breast)] were obtained from the ATCC, whereas A2780 cells (ovarian) was obtained from Sigma-Aldrich. Cells from the ATCC and Sigma were received in 2010. All cells were authenticated before receipt and were propagated for less than 6 months after resuscitation. Cultures are regularly tested for ***Mycoplasma***. All cell lines were cultured in humidified CO_2 atmosphere at 37°C using media recommended by the manufacturer.

Pharmacokinetic and tissue biodistribution study

Five-week-old female NOD/SCID mice were purchased from Charles River Laboratory. The care and treatment of experimental animals were in accordance with the Institutional Animal Care and Use Committee guidelines. Subcutaneous tumors were established by injecting 10 million HT-29 cells into the right flank of mice. When the average tumor volume reached approximately 200 mm^3, mice were randomized into groups ($n = 4$/time point) that received a single intravenous (i.v.) dose of nal-IRI at 5, 10, 20, or 40 mg/kg. Following 1, 4, 8, 24, 48, 72, and 168 hours after a single dose, mice were sacrificed and perfused with PBS before harvest of tumor and other normal tissues.

Antitumor activity studies

Five-week-old female NOD/SCID mice were purchased from Charles River Laboratory. Subcutaneous tumors were established by injecting 10 million HT-29 and SK-ES-1 cells or 5 million A549 cells into the right flank of mice. Tumor growth was measured twice per week by calipers and calculated with formula: width^2 × length × 0.52. When the average tumor volume reached approximately 200 mm^3, mice were randomized into treatment groups ($n = 5$–8/group) that received weekly i.v. dose of PBS (control), free irinotecan (50 mg/kg), or nal-IRI at various doses ranging from 1.25 to 20 mg/kg.

Tumor growth inhibition (TGI) was calculated with formula:

$$\mathrm{TGI}(\%) = \left[1 - \frac{(V_{\mathrm{treated}}(d_{\mathrm{final}}) - V_{\mathrm{treated}}(d_0))}{(V_{\mathrm{control}}(d_{\mathrm{final}}) - V_{\mathrm{control}}(d_0))}\right] \quad \text{(A)}$$

where V_{treated} and V_{control} represent the volumes of tumor at a given time point following treatment with drug or PBS, and d_0 and d_{final} represent first day and final day of treatment, respectively.

Characterizing tumors from cell-line and patient-derived xenografts

The cell line–derived xenografts (HT-29, SK-ES-1, A549, MDA-MB-231, LoVo, AsPC-1, and A2780) were established as described above. The patient-derived tumor models [CTG-0062 (colorectal), CTG-0079 (colorectal), CTG-0252 (ovarian), CTG-0288 (pancreatic), CTG-0158 (lung), and CTG-0283 (pancreatic)] were established by Champions Oncology using their Champions TumorGraft (CTG) technology. When the

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average tumor volume reached approximately 300 mm^3, mice were randomized into treatment groups ($n = 4$/group) that received single i.v. dose of either PBS or nal-IRI at 10 mg/kg. Before tumor collection, intracardial perfusion was performed to remove the blood components from the tumor compartment. Briefly, a butterfly needle (23G) connected to a 10-mL syringe filled with PBS is inserted into the left ventricle. Inferior vena cava is cut and animal is perfused with 10-mL PBS (within 1–2 minutes). The control tumors were harvested 24 hours after PBS administration and used for the irinotecan activation assay, whereas the treated tumors were harvested either 24 or 72 hours following nal-IRI treatment and used for high-performance liquid chromatography (HPLC) analysis.

HPLC quantification of CPT-11 and SN-38

Tumor and normal tissues were analyzed for CPT-11 and SN-38 concentrations using a modification of the method previously described (9). Briefly, tissues were weighed and homogenized for 2 minutes in 20% w/v water using a TissueLyser (Qiagen). The homogenates were extracted by mixing 0.1 mL homogenate with 0.9 mL 1% acetic acid/methanol followed by 10 seconds vortexing and placing at −80°C for 1 hour. The samples were centrifuged at 10,000 rpm for 10 minutes at room temperature and supernatants collected for HPLC analysis (Dionex). The samples and standards (CPT-11 and SN-38) were analyzed using a C18 reverse phase column (Synergi Polar-RP 80A 250 × 4.60 mm 4 μm column). The drug metabolites were eluted running a gradient from 30% acetonitrile; 70% 0.1% TFA/H_2O to 68% acetonitrile; 32% 0.1% TFA/H_2O during a 13 minutes span at a flow rate of 1.0 mL/min. The initial elute composition was restored after 14 minutes and continued for 6 minutes before the next injection. The CPT-11 peak was detected at approximately 7.7 minutes and the SN-38 peak eluted at approximately 8.4 minutes, using an in-line fluorescence detector excited at 372 nm and emitting at 556 nm.

Irinotecan activation assay

Tumor tissue lysates were prepared by homogenizing the tissue in 6% w/v 0.1 M Tris HCL/1% Triton X-100 solution (pH7.5) using a TissueLyser for 2 to 4 minutes. Protein concentration of lysates was measured using the BCA reagent (Thermo Scientific). Lysates (250 μg of protein) were mixed with an equal volume of 10 μmol/L irinotecan and incubated at 37°C. Following 24 hours of incubation the reaction was terminated by adding an equal volume of 1% acetic acid/methanol and samples centrifuged at 10,000 rpm for 15 minutes. The supernatant was processed for HPLC quantification of CPT-11 and SN-38 as described above.

Statistical analysis

The statistical significance of differences between groups was analyzed with the one-way ANOVA test. Results were considered statistically significant at $P < 0.05$. The analysis was performed using GraphPad Prism 6.01.

Model development and simulation

Pharmacokinetic profiles of metabolites in plasma and tumor from free irinotecan and nal-IRI were described by using multi-compartmental models (Fig. 1B). The model equations are explained and summarized in the Supplementary

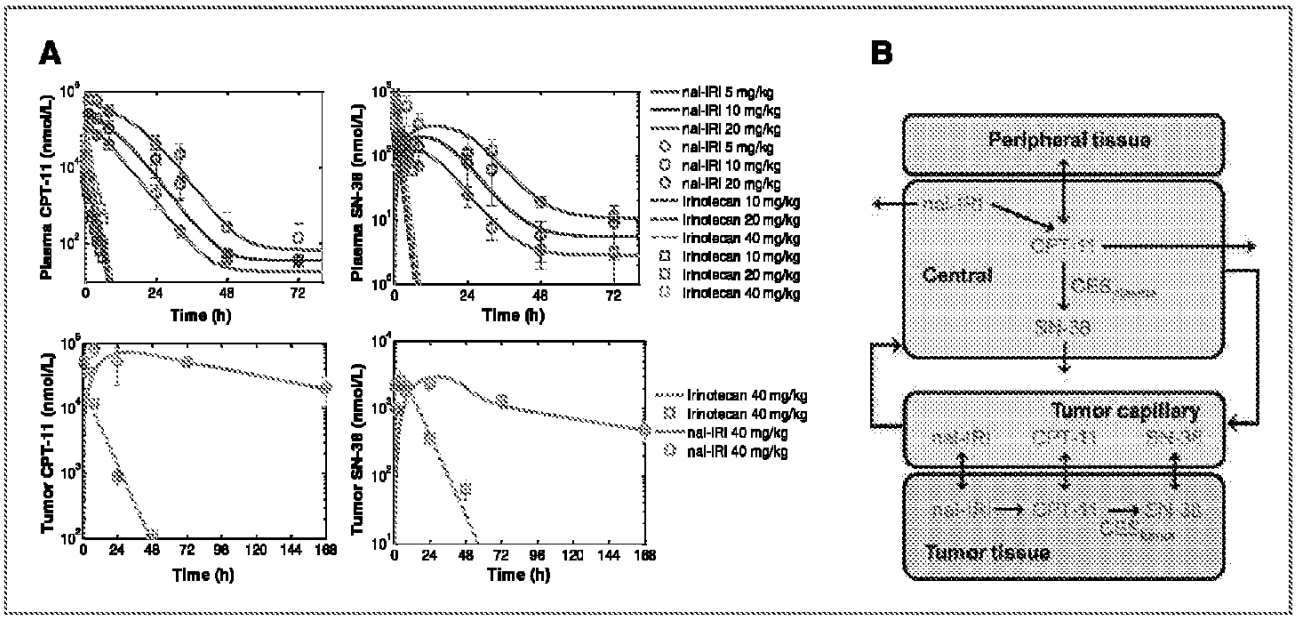

Figure 1. Pharmacokinetic profile of nal-IRI and free irinotecan. A, plasma and tumor PK of nal-IRI were compared with free irinotecan in HT-29 xenograft bearing mice. NOD/SCID mice bearing HT-29 tumors were treated with single i.v. dose of free irinotecan or nal-IRI. Plasma and tumors were collected at various intervals; CPT-11 and SN-38 were measured by HPLC analysis ($n = 4$ animals/time point). Plasma PK data for free irinotecan were taken from Kaneda and colleagues (35). Solid lines represent the model simulations for nal-IRI, whereas dashed lines represent the model simulations for free irinotecan. B, diagram of the mechanistic tumor pharmacokinetic model developed to describe the various steps in metabolism, pharmacokinetics and tumor deposition of nal-IRI.

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Table 1. Summary of model parameters for plasma PK and tumor deposition models

Name	Value	Units	Description	Reference
Plasma PK model parameters				
For free irinotecan				
Cl_{CPT-11}	1.222e−4	L/min	Plasma clearance rate of CPT-11	Estimated
Cl_{SN-38}	2.138e−5	L/min	Plasma clearance rate of SN-38	Estimated
$k_{12,CPT-11}$	8.444e−5	1/min	Rate of CPT-11 transport from plasma to peripheral compartment	Estimated
$k_{21,CPT-11}$	4.213e−2	1/min	Rate of CPT-11 transport from peripheral to plasma compartment	Estimated
$k_{12,SN-38}$	2.656e−5	1/min	Rate of CPT-11 transport from plasma to peripheral compartment	Estimated
$k_{21,SN-38}$	3.44e−4	1/min	Rate of CPT-11 transport from peripheral to plasma compartment	Estimated
$V_{max,CES,p}$	2.263e−1	nmol/min	Maximum rate coefficient for CES enzyme in plasma compartment	Estimated
$K_{m,CES,p}$	2.67e5	nmol/L	Michaelis-Menten constant for CES enzyme in plasma compartment	Estimated
V_p	9.46e−5	L	Volume of plasma compartment	Estimated
V_{ph}	0.02	L	Volume of peripheral compartment	Fixed (14)
For nal-IRI				
$Cl_{nal-IRI}$	1.87e−7	L/min	Plasma clearance rate of nal-IRI	Estimated
Cl_{CPT-11}	1.634e−5	L/min	Plasma clearance rate of CPT-11	Estimated
Cl_{SN-38}	2.957e−6	L/min	Plasma clearance rate of SN-38	Estimated
$k_{12,CPT-11}$	1.619e−4	1/min	Rate of CPT-11 transport from plasma to peripheral compartment	Estimated
$k_{21,CPT-11}$	5.349e−7	1/min	Rate of CPT-11 transport from peripheral to plasma compartment	Estimated
$V_{max,Release,p}$	8.443e−6	nmol/min	Maximum rate coefficient for CPT-11 release from nal-IRI in plasma compartment	Estimated
$K_{m,Release,p}$	2.04	nmol/L	Michaelis-Menten constant for CPT-11 release from nal-IRI in plasma compartment	Estimated
$V_{max,CES,p}$	5.943e−2	nmol/min	Maximum rate coefficient for CES enzyme in plasma compartment	Estimated
$K_{m,CES,p}$	1.198e−5	nmol/L	Michaelis-Menten constant for CES enzyme in plasma compartment	Estimated
V_p	1.12e−3	L	Volume of plasma compartment	Estimated
V_{ph}	0.02	L	Volume of peripheral compartment	Fixed (14)
Tumor deposition model parameters				
Q_{tumor}	2.119e−6	L/min	Blood flow rate to tumor	(14)
$PS_{nal-IRI}$	7.858e−5	L/min/kg	Tissue permeability coefficient of nal-IRI	Estimated
PS_{CPT-11}	1.851e−3	L/min/kg	Tissue permeability coefficient of CPT-11	Estimated
PS_{SN-38}	2.687e−2	L/min/kg	Tissue permeability coefficient of SN-38	Estimated
$\sigma_{nal-IRI}$	3.181e−3		Tissue-capillary partition coefficient of nal-IRI	Estimated
σ_{CPT-11}	5.24e−1		Tissue-capillary partition coefficient of CPT-11	Estimated
σ_{SN-38}	2.109e−1		Tissue-capillary partition coefficient of SN-38	Estimated
$k_{Release,t}$	1.681e−4	1/min	Rate coefficient for CPT-11 release from nal-IRI in tumor tissue compartment	Estimated
$V_{max,CES,t}$	2.17e−2	nmol/min	Maximum rate coefficient for CES enzyme in tumor tissue compartment	Estimated
$K_{m,CES,t}$	2.3e−6	nmol/L	Michaelis-Menten constant for CES enzyme in tumor tissue compartment	Estimated
V_{cap}	7e−7	L	Volume of tumor capillary compartment	(14)
V_t	1e−5	L	Volume of tumor tissue compartment	Fixed

Data. The models were built and implemented using Simbiology toolbox in MATLAB 8.2 (The MathWorks).

Results

nal-IRI displays a prolonged exposure in both plasma and tumor compared with free irinotecan

The pharmacokinetic profiles of the prodrug CPT-11 and its active metabolite SN-38 were measured in plasma and tumors following administration of either free irinotecan or nal-IRI (Fig. 1A). At similar doses of both free irinotecan and nal-IRI, the CPT-11 and SN-38 plasma levels cleared rapidly from circulation within 8 hours after free irinotecan injection, whereas the levels of CPT-11 and SN-38 following nal-IRI administration were persistent and remained in circulation for over 50 hours. An approximately 10-fold higher plasma CPT-11 peak level was observed with nal-IRI as compared with free irinotecan. However, the plasma peak level of SN-38 achieved with nal-IRI was 10-fold lower compared with free irinotecan, probably due to the ability of the lipid bilayer to protect the conversion of prodrug CPT-11 to SN-38 by the systemic CES enzyme present in mouse models (17). Administration of free irinotecan resulted in the clearance of greater than 90% of CPT-11 from tumors within 24 hours; however, following nal-IRI administration, CPT-11 levels persisted above

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10,000 nmol/L levels for 168 hours. Similar peak levels of SN-38 were achieved with both free irinotecan and nal-IRI in HT-29 tumors, though a prolonged SN-38 exposure for up to 168 hours (measured as the AUC from 0 to 168 hours) was achieved with nal-IRI as compared with less than 48 hours tumor exposure with free irinotecan. In summary, CPT-11 and SN-38 were still present in tumors at 168 hours following nal-IRI administration, though both CPT-11 and SN-38 had cleared from plasma.

Tumor SN-38 duration drives *in vivo* activity

We developed a mechanistic PK model to identify the determinants that may differentiate the plasma and tumor PK profiles between free irinotecan and nal-IRI (Fig. 1B). The experimental PK data were used to estimate the optimal model parameters (Table 1) fitting the model simulations within the standard deviations of ***in vivo*** PK profiles of both CPT-11 and SN-38 (Fig. 1A). As the ***in vitro*** cytotoxic effects of irinotecan on tumor cells is dependent on the concentration and the time of exposure of cells to active metabolite SN-38 (7, 8), we sought to understand if the overall plasma and tumor SN-38 exposure predicts the ***in vivo*** activity of both nal-IRI and free irinotecan. The trained model determined that a 5-fold higher dose of free irinotecan (50 mg/kg) was required to achieve similar SN-38 exposure in both plasma and tumor as compared with nal-IRI (10 mg/kg; Fig. 2A). The TGI of HT-29 xenograft model at these equal exposure doses, was significantly greater with nal-IRI (~110%) treatment as compared with free irinotecan (~40%), despite the 5-fold lower total dose administered (*, $P < 0.05$,

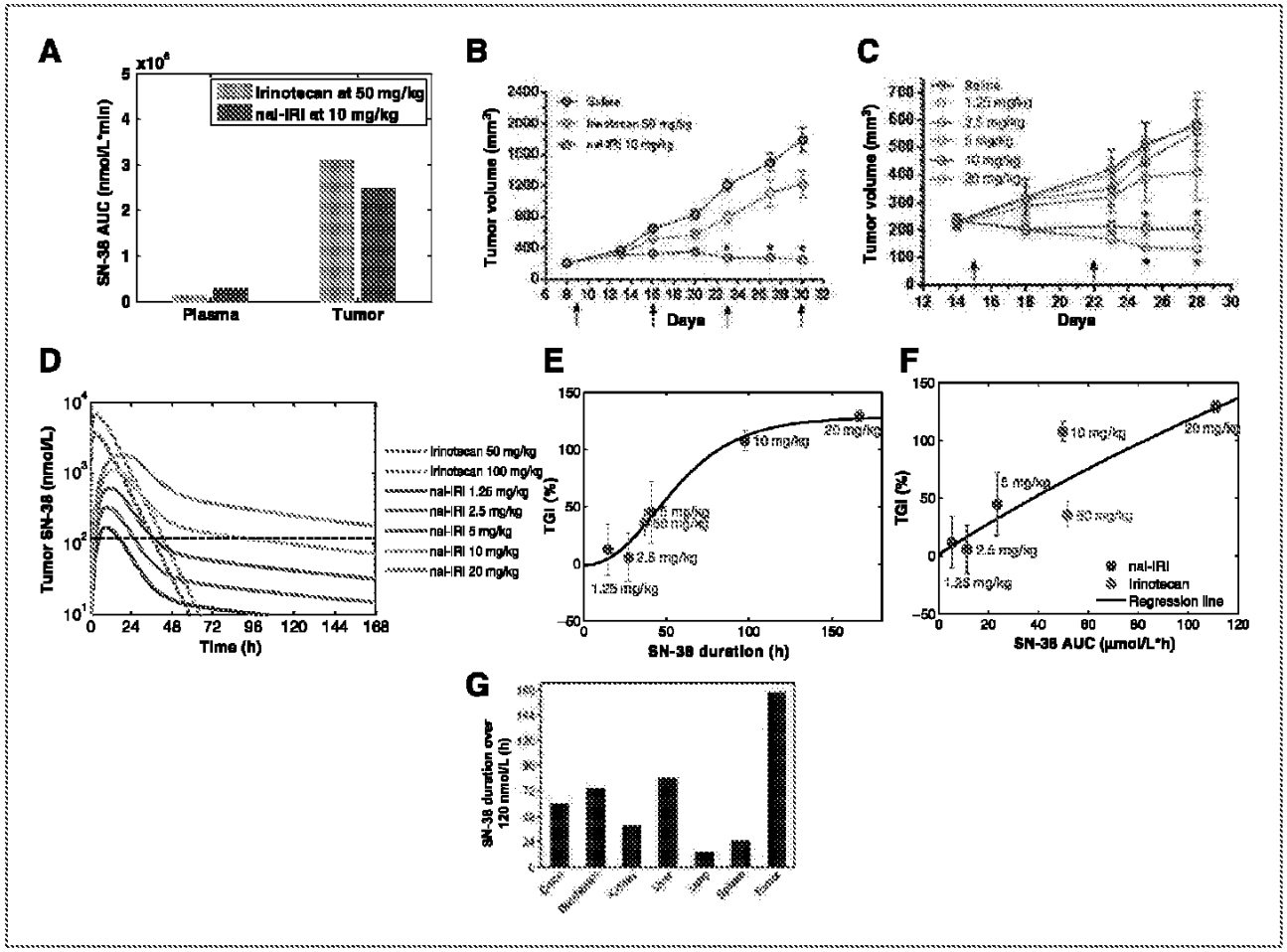

Figure 2. Relation of nal-IRI *in vivo* activity to tumor SN-38 duration. A, model predictions for similar SN-38 AUC in plasma and tumor following free irinotecan (50 mg/kg) and nal-IRI (10 mg/kg) administration. B, tumor response observed in HT-29 xenograft following weekly administration (arrows) of 50 mg/kg free irinotecan and 10 mg/kg nal-IRI ($n = 8$/group). The tumor volumes for nal-IRI (10 mg/kg) were significantly lower (*, $P < 0.05$) compared with saline and irinotecan groups (one-way ANOVA test). C, tumor response in HT-29 xenografts following weekly administration (arrows) of various nal-IRI doses ($n = 5$/group). The tumor volumes for nal-IRI (10 mg/kg) and nal-IRI (20 mg/kg) groups were significantly lower (*, $P < 0.05$) compared with saline tumors on day 25 and day 28 (one-way ANOVA test). D, model simulations were used to compare tumor SN-38 concentration following the administration of varying doses of free irinotecan or nal-IRI. Black dashed line represents threshold concentration of 120 nmol/L to determine tumor SN-38 duration. E and F, TGI(%) achieved by nal-IRI and free irinotecan treatment in HT-29 xenografts were compared with the tumor SN-38 duration above 120 nmol/L (E) and SN-38 AUC (F) at varying doses of nal-IRI or free irinotecan. Solid lines represent nonlinear regression lines based on five parameter logistic curve fitting. G, the SN-38 duration over a threshold of 120 nmol/L was computed from the pharmacokinetic profiles of SN-38 in tumor and normal tissues following 20 mg/kg of nal-IRI.

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one-way ANOVA test; Fig. 2B). In addition, other studies have shown no additional HT-29 TGI at the maximum tolerated dose of free irinotecan (100 mg/kg; ref. 18). To identify a dose level of nal-IRI that gave comparable ***in vivo*** activity to 50 mg/kg free irinotecan, we performed a dose escalation study in the HT-29 xenograft model (Fig. 2C). nal-IRI at 5 mg/kg showed partial inhibition of tumor growth (~40% TGI) that was comparable with 50 mg/kg free irinotecan, whereas 10 mg/kg and 20 mg/kg nal-IRI showed significant (*, $P < 0.05$, one-way ANOVA test) TGI compared with saline (~110%–130% TGI). Furthermore, we have previously tested control liposomes (that have comparable composition with nal-IRI except for the absence of irinotecan, the active pharmaceutical ingredient) and did not observe any TGI (data not shown).

The intratumor SN-38 concentrations achieved from 50 to 100 mg/kg doses of free irinotecan and 1.25, 2.5, 5, 10, and 20 mg/kg doses of nal-IRI were then simulated using the trained mechanistic PK model (Fig. 2D). Although a nal-IRI dose of 5 mg/kg achieved similar TGI as 50 mg/kg free irinotecan, the tumor SN-38 AUC and peak levels were approximately 2-fold and 6-fold lower respectively for nal-IRI as compared with free irinotecan. Furthermore, we noted at these doses both drugs were able to maintain the tumor SN-38 concentration above 120 nmol/L for the same duration of approximately 40 hours. To determine if the tumor SN-38 concentration impacts ***in vivo*** activity, we used the tumor SN-38 concentration of 120 nmol/L as a threshold. We also determined the duration for which the various doses of nal-IRI or free irinotecan could maintain the tumor SN-38 concentration above 120 nmol/L, hereon referred to as "tumor SN-38 duration." A sigmoidal relationship between TGI (%) and tumor SN-38 duration (Fig. 2E) was observed for both nal-IRI and free irinotecan ($R^2 = 0.62$). However, when comparing TGI (%) with tumor SN-38 AUC (Fig. 2F) the relationship was less significant ($R^2 = 0.45$), due to the lower TGI (%) achieved by 50 mg/kg free irinotecan compared with 10 mg/kg nal-IRI. We also observed longer SN-38 duration in tumors (>100 hours) compared with normal tissues (<72 hours; Fig. 2G and Supplementary Fig. S1).

Identification of liposome tumor permeability and local tumor activation as critical determinants for tumor SN-38 duration

A local sensitivity analysis on the model parameters was performed to identify processes impacting the tumor SN-38 duration (Supplementary Data). In response to the administration of free irinotecan (50 mg/kg), the tumor SN-38 duration was relatively insensitive to most model parameters (Fig. 3A), suggesting the inability of free irinotecan to modulate it. In contrast, several model parameters were found to significantly impact tumor SN-38 duration following the administration of nal-IRI (10 mg/kg; Fig. 3B). The sensitive parameters for nal-IRI can be classified into three different categories: (i) PK, rate of breakdown of liposomes in blood (Release rate in blood, $V_{max,Release,p}$), (ii) activation of prodrug CPT-11 to SN-38 by CES enzyme (CES activity in tumor; $V_{max,CES,t}$ and blood; $V_{max,CES,p}$), and (iii) liposome uptake within tumors, that is, nal-IRI tumor deposition (nal-IRI tumor permeability, $PS_{nal\text{-}IRI}$). Among these parameters, the release rate in plasma negatively affected tumor SN-38 duration due to a decrease in the overall systemic exposure of nal-IRI. CES enzyme activity, particularly from tumor CES (local tumor activation of irinotecan) and nal-IRI permeability (tumor deposition), positively affected the tumor SN-38 duration. To assess the identifiability of parameter estimates, log likelihood profiling was performed for the sensitive parameters, $V_{max,CES,t}$ and $PS_{nal\text{-}IRI}$ (19). The confidence intervals suggested that both parameters were precisely estimated (Supplementary Fig. S2).

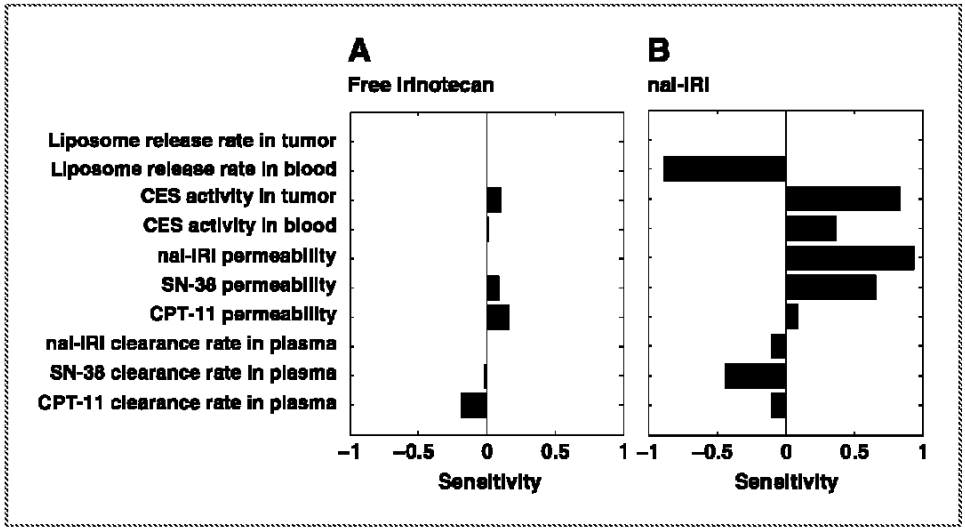

Figure 3. Model parameters impacting tumor SN-38 duration. Sensitivity analyses for free irinotecan (A) and nal-IRI (B) were performed on key model parameters that are responsible for plasma clearance, tissue deposition and metabolic reactions. Parameters whose values were not estimated in this study, including compartment volumes and tumor blood flow, were excluded from the analysis. The doses of free irinotecan (50 mg/kg) and nal-IRI (10 mg/kg) that achieved similar SN-38 plasma and tumor exposure were used for sensitivity analysis. The model parameters were modulated by 10% and their effect on tumor SN-38 duration was determined as a sensitivity index (Supplementary Equation S6).

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Biologic variability and simulated perturbation of nal-IRI tumor deposition and local activation

To determine the biologic relevance of these sensitive parameters toward driving tumor SN-38 duration (namely nal-IRI tumor deposition and local tumor activation of irinotecan), the parameters were measured in a panel of 13 xenograft models. We used the total CPT-11 concentrations in tumors as a surrogate for nal-IRI tumor deposition as model simulations based on nal-IRI pharmacokinetics showed that majority of CPT-11 in plasma and tumor was encapsulated and protected within the liposomes and less than 10% was available as free CPT-11 (Supplementary Fig. S3). The intratumor concentrations of CPT-11 varied substantially across the tumor panel (Fig. 4A). The tumor models from cell-lines displayed overall higher levels of prodrug CPT-11 deposition (from 5,000–15,000 ng/g) as compared with patient-derived tumor models (1,000–2,000 ng/g). In addition, a high degree of variability was observed between individual tumors within the same xenograft model (66% average coefficient of variation). Model simulations were used to test the effect of altering nal-IRI tumor deposition on tumor SN-38 duration (Fig. 4B). By decreasing the nal-IRI permeability parameter to zero, which simulates an impermeable tumor microenvironment, the tumor SN-38 duration of approximately 100 hours achieved

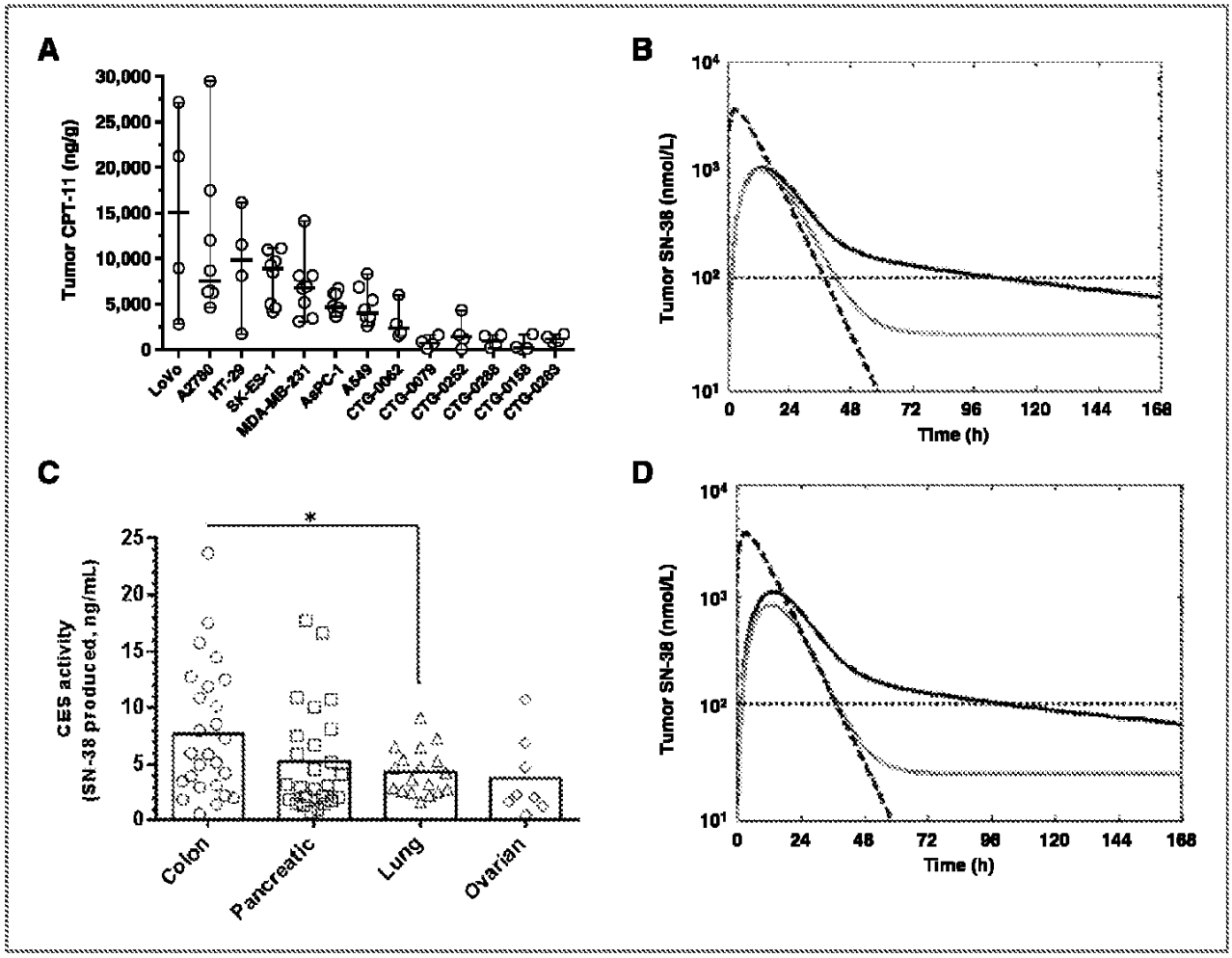

Figure 4. ***In vivo*** variability in nal-IRI tumor deposition and local activation. A, intratumor CPT-11 concentrations were measured across cell-line–derived (HT-29, SK-ES-1, A549, MDA-MB-231, LoVo, AsPC-1, and A2780) and patient-derived (CTG-0062, CTG-0079, CTG-0252, CTG-0288, CTG-0158, and CTG-0283) tumor models. Tumor-bearing mice were administered a single i.v. dose of 10 mg/kg nal-IRI and tumors excised 24 hours later. CPT-11 concentrations were determined in the tumor lysates using HPLC analysis as described in Materials and Methods ($n = 4$–8 tumors/model). B, the effect of nal-IRI permeability on tumor SN-38 concentrations was simulated by reducing the nal-IRI permeability parameter $PS_{nal\text{-}IRI}$ to zero. The simulated tumor SN-38 concentrations were compared at the equal exposure doses of 10 mg/kg nal-IRI and 50 mg/kg free irinotecan. Black solid line, nal-IRI (10 mg/kg) with base $PS_{nal\text{-}IRI}$. Gray solid line, nal-IRI (10 mg/kg) with zero $PS_{nal\text{-}IRI}$. Black dashed line, free irinotecan (50 mg/kg). Dotted line, threshold concentration of 120 nmol/L CES activity (C) for 80 patient-derived xenograft tumors across different indications was determined using ***ex vivo*** irinotecan activation assay. Tumor lysates (250 μg of protein) from untreated mice was incubated with free irinotecan (5 μmol/L) for 24 hours at 37°C and the amount of SN-38 produced was measured with HPLC analysis (*, $P < 0.05$; *t* test). D, the effect of knocking out tumor CES activity on tumor SN-38 duration was simulated by reducing the tumor CES parameter $V_{max,CES,t}$ to zero. The simulated tumor SN-38 concentrations were compared at the equal exposure doses of 10 mg/kg nal-IRI and 50 mg/kg free irinotecan. Black solid line, nal-IRI 10 mg/kg with base $V_{max,CES,t}$. Gray solid lines, nal-IRI 10 mg/kg with zero $V_{max,CES,t}$. Black dashed line, free irinotecan 50 mg/kg with base $V_{max,CES,t}$. Dotted line, threshold concentration of 120 nmol/L.

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with 10 mg/kg nal-IRI was substantially reduced to approximately 50 hours and approached the levels observed with 50 mg/kg free irinotecan. Taken together, these results suggest that the tumor deposition of nal-IRI is highly tumor specific and will dramatically impact tumor SN-38 duration.

To determine the degree to which local tumor activation of irinotecan varied in human tumors, we measured CES activity using an ***ex vivo*** assay in a panel of 80 patient-derived tumors. The tumor lysates varied in their ability to activate prodrug irinotecan and produce SN-38 (1–25 ng/mL SN-38 produced), suggesting a high degree of variability in local tumor activation of irinotecan across indications. A significant difference in local tumor activation of irinotecan was observed between colon and lung tumors ($P < 0.05$). However, there was no significant difference between other indications, which may be due to high variability observed within each indication (Fig. 4C). The impact of varying the tumor CES activity on tumor SN-38 duration was evaluated by simulating a knockout of tumor CES enzyme (Fig. 4D). In the absence of local tumor activation, tumor SN-38 duration with nal-IRI (10 mg/kg) decreased from approximately 100 to 40 hours, similar to that achieved by free irinotecan (50 mg/kg).

nal-IRI tumor deposition and local activation collectively predict tumor SN-38 duration

The relative contribution of nal-IRI tumor deposition and local tumor activation on tumor SN-38 duration was evaluated using model simulations. On the basis of the findings from the sensitivity analysis (Fig. 3B), nal-IRI permeability ($PS_{nal\text{-}IRI}$) and tumor CES activity ($V_{max,CES,t}$) values were used to create a map relating these parameters to tumor SN-38 duration following nal-IRI administration (Fig. 5A). Model simulations predicted a concave relationship, where the tumor SN-38 duration is dependent upon both the tumor permeability and the tumor CES activity. The tumor SN-38 duration could be increased by either increasing the $PS_{nal\text{-}IRI}$ or $V_{max,CES,t}$ (white arrows) and the maximum tumor SN-38 duration of 168 hours was only reached with CES activity at 0.025 nmol/min and tumor permeability at 1.5E−4 L/min/kg.

To experimentally test the model predictions, we used the same panel of 13 xenograft models to measure the tumor concentrations of CPT-11 (as a surrogate for tumor deposition, Supplementary Fig. S4A); tumor SN-38 concentrations (as a surrogate for tumor SN-38 duration; Supplementary Fig. S4B) and CES activity (for local tumor activation of irinotecan). The experimental data supported the model simulations, confirming that the SN-38 concentration within tumors was dependent on both the tumor CPT-11 concentration and tumor CES activity (Fig 5B). All tumor models with high CPT-11 concentration >2,000 ng/mL or high CES activity > 5 ng/mL displayed high tumor SN-38 concentrations ("red") ranging from 25 to 125 ng/mL (Supplementary Table S2). In certain tumor models, one of the parameters contributed predominantly toward higher SN-38 concentrations (black arrows). A2780 and SK-ES-1 tumors displayed high tumor SN-38 concentrations of 97 ng/mL and 127 ng/mL respectively (Supplementary Table S2), which was mainly due to high CPT-11 concentrations (>2,000 ng/mL), whereas in other tumor models (CTG-0062 and AsPC-1) the CES activity (>5 ng/mL) was the dominant factor contributing toward high tumor SN-38 concentrations. Further tumor models with the lowest tumor SN-38 concentrations ranging from 5 to 12 ng/mL ("blue"), including several patient-derived tumor models (boxed area) also displayed lower tumor CPT-11 concentrations (<2,000 ng/mL) and CES enzyme activity (<5 ng/mL).

Tumor SN-38 duration correlates with nal-IRI *in vivo* activity

In vivo tumor response studies were performed in three tumor models in which different tumor SN-38 durations had been observed (as indicated by tumor SN-38 concentration at 72 hours) to determine the impact of tumor SN-38 duration on ***in vivo*** activity of nal-IRI. The tumor volumes observed for both HT-29 (Fig. 6A) and SK-ES-1 (Fig. 6B) models were significantly

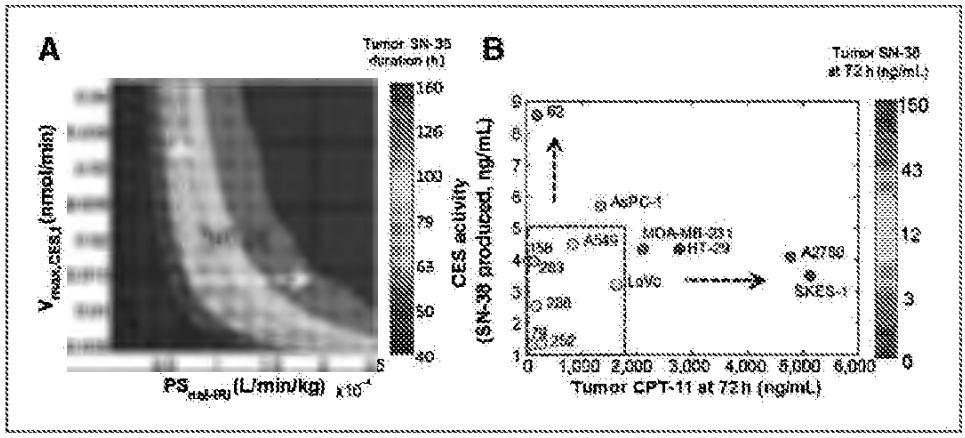

Figure 5. nal-IRI tumor deposition and local activation impacts tumor SN-38 duration. A, the effect of changing tumor CES activity and nal-IRI permeability parameters (arrows) on tumor SN-38 duration (color-coded in hours) in tumors was simulated. The optimal parameter values for HT-29 were marked with the symbol "*". B, experimental data in tumor xenograft models showing the impact of tumor CPT-11 and CES activity on tumor SN-38 concentrations. Tumor CES activity (as surrogate for local tumor activation of irinotecan) and tumor CPT-11 concentration at 72 hours (as surrogate for tumor deposition) for different xenograft models were plotted and color-coded on the basis of their SN-38 concentrations in the tumor 72 hours after nal-IRI (each data point represents median of $n = 4$–8 tumors). Dotted arrows, dependence of tumor SN-38 concentrations on tumor CPT-11 concentration and CES activity.

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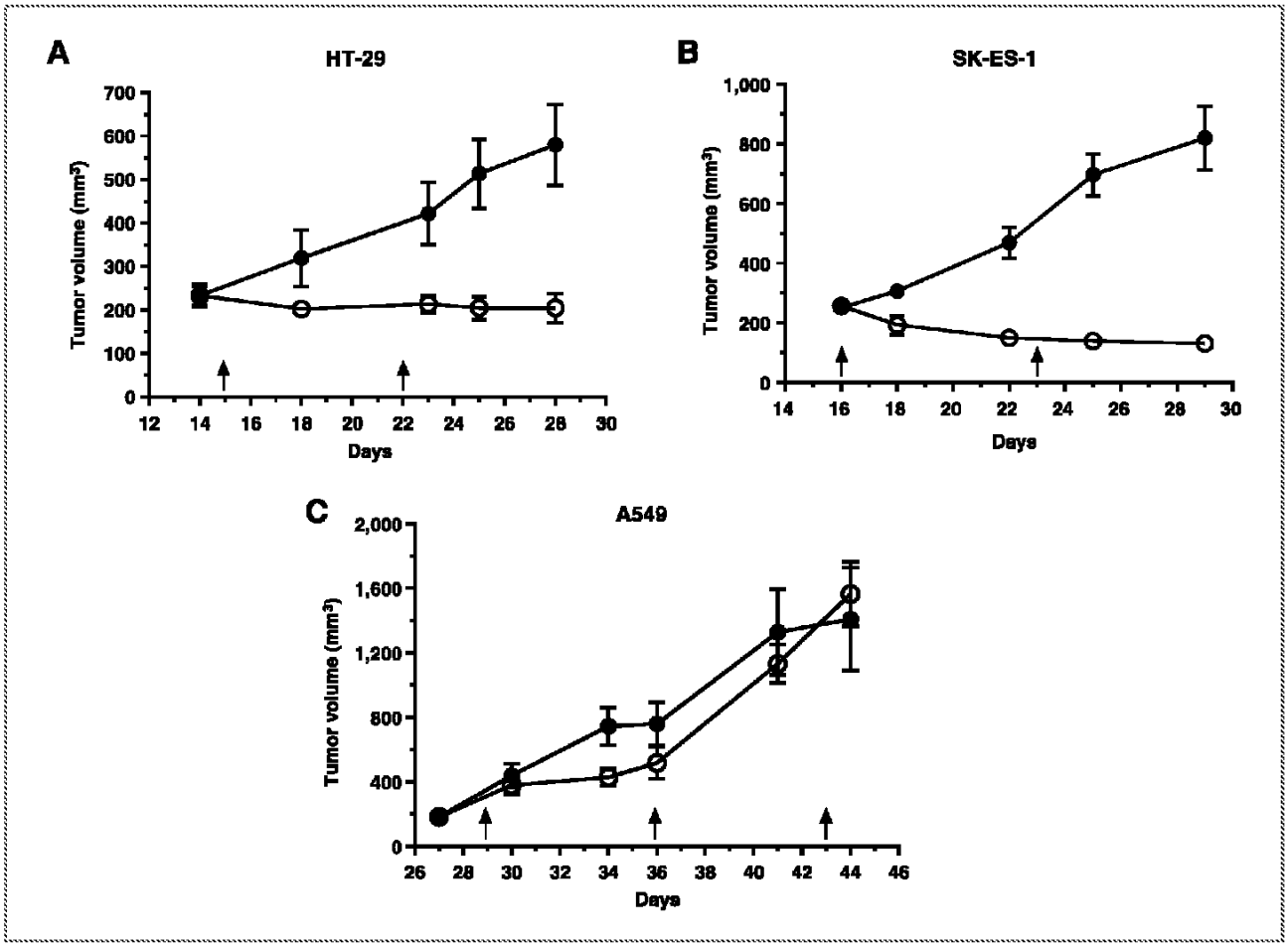

Figure 6. ***In vivo*** tumor response for nal-IRI. NOD/SCID mice were inoculated with HT-29; colon (A), SK-ES-1; Ewing's (B) and A549; lung (C) cell lines. Tumor-bearing mice were randomized when the tumor volume was approximately 200 mm^3. Each group received weekly i.v. dose (arrows) of either saline (●) or 10 mg/kg dose of nal-IRI (○). Tumor volumes were measured twice per week (n = 5–10 animals/group).

lower ($P < 0.05$) following 10 mg/kg nal-IRI as compared with untreated tumors. In both these models, tumor regression was observed immediately after the first dose and was sustained through the course of the study. A549 tumors achieved lower SN-38 tumor levels (Fig. 4A) and did not respond to nal-IRI treatment (Fig. 6C). Interestingly, both A549 and HT-29 cells displayed similar ***in vitro*** sensitivity to SN-38 with IC_{50} values of 53 and 44 nmol/L, respectively (20). In summary, nal-IRI induced stronger responses (~100%TGI) in tnmor models that had higher tumor SN-38 duration (>~100 hours).

Discussion

The nal-IRI formulation dramatically alters the pharmacologic properties of irinotecan as well as its active metabolite, SN-38 (9). In this study, we identified a pharmacologic parameter—namely, tumor SN-38 duration—as a driver of irinotecan-based ***in vivo*** activity and propose biomarkers that can impact tumor SN-38 duration achieved by nal-IRI. Our study indicates that nal-IRI can completely inhibit tumor growth compared with free irinotecan, despite administering doses that achieve similar SN-38 exposure (measured as the AUC). Instead, the duration of prolonged exposure of SN-38 within tumors achieved by nal-IRI was shown to be a major pharmacologic determinant for ***in vivo*** activity in mice.

Several studies have shown improved ***in vitro*** cytotoxic activity of SN-38 when cells are exposed to drug for longer duration (21). The ***in vitro*** cell doubling time for HT-29 cells is approximately 20 hours (21), whereas ***in vivo*** the tumor volume doubles (Fig. 2B) at a slower rate (~8–9 days). In addition, at a given time only 35 to 50% of cells are in the S-phase of cell cycle wherein the maximum cytotoxicity of free irinotecan has been observed (21). Thus, to exert maximum cytotoxic effects across different cell-cycle phases, the cells have to be exposed to free irinotecan across multiple cell cycles. Our ***in vivo*** study confirms these findings as the free irinotecan is rapidly cleared from plasma and tumor tissue (tumor SN-38 dnration of approximately 40 hours), thereby not allowing sufficient time for tumor cells to be exposed to SN-38 (for only 2 cell-cycle doubling time) as compared with more than 5 cell-cycle doubling times with nal-IRI (tumor SN-38 duration for >100 hours). Thus the extended exposure of tumor cells to SN-38, which is achieved by nal-IRI, can contribute toward the enhanced cytotoxicity as compared with free irinotecan.

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We observed higher tumor concentrations of CPT-11 and SN-38 at 168 hours following administration of nal-IRI. In contrast, the peak plasma concentrations of SN-38 was lower with nal-IRI as compared with free irinotecan, suggesting that most of the CPT-11 from nal-IRI remains inside the liposomes and is protected from systemic conversion as described with free irinotecan (17). In addition, prolonged SN-38 duration from nal-IRI administration was observed only in tumors and much less in normal tissues, suggesting that toxicity might not be exacerbated by nal-IRI treatment. The preferentially accumulation of nal-IRI in tumors as compared with normal tissues can be attributed to the EPR effect, where the leaky vasculature in tumor facilitates the extravasation of liposomal nanoparticles and the defective lymphatic drainage helps increase the retention within tumor (1, 2). Thus, with the EPR effect, nal-IRI creates a large depot of CPT-11 only in tumors thereby prolonging tumor SN-38 duration. In contrast, free irinotecan can easily be transported in and out of the tissues with a short plasma half-life, resulting in minimal SN-38 duration in tumors.

The enhanced ***in vivo*** activity of nal-IRI as compared with free irinotecan was attributed to the ability of nal-IRI to extend the tumor SN-38 duration. Sensitivity analysis identified two key determinants that impact the ability of nal-IRI to extend tumor SN-38 duration—(i) nal-IRI tumor deposition, as measured by the extent of prodrug CPT-11 deposition within tumors and (ii) nal-IRI local activation, from prodrug CPT-11 to SN-38 facilitated by the local tumor CES enzyme. The experimental data, in this study supported the importance of each of these determinants. We observed high degree of variability in the overall nal-IRI tumor deposition across the 13 xenograft models that were tested. Several studies have highlighted a role for tumor permeability, tumor perfusion, and stromal matrix in limiting the delivery of therapeutic agents into tumors (22). In our model simulations, when the nal-IRI tumor permeability was decreased to zero, the benefit of higher tumor SN-38 duration with nal-IRI was negatively impacted and reduced to levels simulated for free irinotecan. We also observed that the tumors with lower nal-IRI deposition had considerable lower SN-38 tumor levels. These data are consistent with other findings suggesting that a dense tumor stroma can impede drug permeability and limit drug delivery within tumors (23, 24).

Use of tumor CES activity as a cellular parameter for predicting free irinotecan response had limited success both in preclinical (25, 26) and clinical studies.(27). Through the sensitivity analysis performed in this study, we identified CES activity as a critical parameter for nal-IRI activity. Tumor models that displayed high ability to activate CPT-11, achieved high tumor SN-38 concentrations despite limited deposition of CPT-11, thus suggesting the importance of local tumor CES enzyme expression in facilitating longer SN-38 exposure following nal-IRI administration. In fact, others have shown that ***in vitro*** and ***in vivo*** activity of free irinotecan can be enhanced by overexpressing of CES enzyme in tumor cells (28, 29). In addition to tumor cells expressing CES enzyme (30), other components of the extracellular matrix such as tumor-associated macrophages (TAMs) express CES1 enzyme and play a role in CPT-11 activation (31). In fact, we performed ***in vitro*** studies that confirmed the ability of TAMs to hydrolyze CPT-11 to SN-38 (Supplementary Fig. S5). Thus our data suggests the extended tumor PK achieved by nal-IRI provides high local depot of prodrug CPT-11 for prolonged time, thus allowing for activation by tumor CES enzymes. Collectively our data provides rational for investigating tumor CES enzyme activity as a potential marker for nal-IRI activity.

Pharmacogenetic and pharmacodynamic markers such as TOP1 have shown limited correlations with free irinotecan response (6, 32–34). In addition to the intrinsic sensitivity of tumor cells to SN-38, our data indicate that the duration for which tumor cells are exposed to SN-38 (tumor SN-38 duration) also plays a critical role in driving treatment response to irinotecan. Tumor models with extended SN-38 duration (HT-29, SK-ES-1) showed robust ***in vivo*** response to nal-IRI, whereas A549 with shorter tumor SN-38 duration did not respond to therapy. The fact that ***in vitro*** sensitivity of both HT-29 and A549 to SN-38 is very similar (20) corroborates the finding that the duration of SN-38 is driving the tumor response.

In conclusion, our data demonstrate that nal-IRI enhances the pharmacokinetic profile of tumor SN-38, prolonging tumor exposure to SN-38 compared with free irinotecan, and therefore has the potential for therapeutic effect in human cancers. Liposome permeability and CES activity were the critical factors that emerged from model simulation of tumor SN-38 duration, which were experimentally shown to vary across and within tumor indications. Thus, translational research exploring the utility of tumor liposome permeability and local activation of irinotecan as biomarkers for nal-IRI clinical activity is warranted.

Disclosure of Potential Conflicts of Interest

D.C. Drummond and J.B. Fitzgerald have ownership interest (including patents) in Merrimack Pharmaceuticals, Inc. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: A.V. Kalra, J. Kim, S.G. Klinz, D.C. Drummond, U.B. Nielsen, J.B. Fitzgerald
Development of methodology: A.V. Kalra, J. Kim, S.G. Klinz
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.V. Kalra, N. Paz, J. Cain
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.V. Kalra, J. Kim, D.C. Drummond, J.B. Fitzgerald
Writing, review, and/or revision of the manuscript: A.V. Kalra, J. Kim, S.G. Klinz, D.C. Drummond, U.B. Nielsen, J.B. Fitzgerald
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Kim, N. Paz
Study supervision: A.V. Kalra, J.B. Fitzgerald

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Cancer Research

Preclinical Activity of Nanoliposomal Irinotecan Is Governed by Tumor Deposition and Intratumor Prodrug Conversion

AU Ashish V. Kalra, Jaeyeon Kim, Stephan G. Klinz, Nancy Paz, Jason Cain, Daryl C. Drummond, Ulrik B. Nielsen, and Jonathan B. Fitzgerald

Abstract

A major challenge in the clinical use of cytotoxic chemotherapeutics is maximizing efficacy in tumors while sparing normal tissue. Irinotecan is used for colorectal cancer treatment but the extent of its use is limited by toxic side effects. Liposomal delivery systems offer tools to modify pharmacokinetic and safety profiles of cytotoxic drugs. In this study, we defined parameters that maximize the antitumor activity of a nanoliposomal formulation of irinotecan (nal-IRI). In a mouse xenograft model of human colon carcinoma, nal-IRI dosing could achieve higher intratumoral levels of the prodrug irinotecan and its active metabolite SN-38 compared with free irinotecan. For example, nal-IRI administered at doses 5-fold lower than free irinotecan achieved similar intratumoral exposure of SN-38 but with superior antitumor activity. Tumor response and pharmacokinetic modeling identified the duration for which concentrations of SN-38 persisted above a critical intratumoral threshold of 120 nmol/L as determinant for antitumor activity. We identified tumor permeability and carboxylesterase activity needed for prodrug activation as critical factors in achieving longer duration of SN-38 in tumors. Simulations varying tumor permeability and carboxylesterase activity predicted a concave increase in tumor SN-38 duration, which was confirmed experimentally in 13 tumor xenograft models. Tumors in which higher SN-38 duration was achieved displayed more robust growth inhibition compared with tumors with lower SN-38 duration, confirming the importance of this factor in drug response. Overall, our work shows how liposomal encapsulation of irinotecan can safely improve its antitumor activity in preclinical models by enhancing accumulation of its active metabolite within the tumor microenvironment. *Cancer Res; 1–11. ©2014 AACR.*

Introduction

Liposomal carriers have become clinically accepted in cancer therapy as delivery systems that can enhance the utility of existing anticancer drugs (1). The potential benefits of these macromolecular carriers include overcoming solubility issues for certain drug classes, protecting the drug from unwanted metabolism and extending the residence time in plasma and tissue. In particular, liposomes tend to preferentially accumulate in tumors as a result of an enhanced permeability and retention (EPR) effect. The EPR effect is attributed to the abnormal tumor vasculature permitting extravasation of macromolecules, as well as impaired lymphatic drainage that promote the retention of these molecules within the tumor microenvironment, thereby providing sustained release at the tumor site mimicking a metronomic dosing (2). Increased tumor deposition via the EPR effect may also prevent drug resistance by overcoming the activity of multidrug resistant proteins (3, 4) and may offer possible means of improving safety aspects by reducing systemic exposure relative to tumor exposure (5). There are potential pharmacologic advantages of the EPR effect, particularly for antineoplastic agents that have to engage their target over a longer time period or have little binding activity; an example is drugs of the camptothecin class with topoisomerase I enzyme (TOP1) as the primary target.

Irinotecan (CPT-11), a clinically approved camptothecin, is a prodrug that is activated by carboxylesterase (CES) enzymes, present primarily in liver and colon tissue to the active form, SN-38. (In the article, CPT-11 is used when referring to the prodrug levels in plasma or tumor samples following either free irinotecan or nal-IRI administration; SN-38 is used when referring to the active metabolite of CPT-11.) The active SN-38 can be subsequently inactivated through glucuronidation by members of the UDP glucuronosyltransferase family (6). The principal mechanism of action leading to cell death is through DNA damage after replication-fork collisions with transient drug-TOP1 cleavage complexes, thus emphasizing the time of drug exposure as important driver for cytotoxicity of camptothecins (7, 8). Recently, we described the development of a novel nanoliposomal formulation of irinotecan, nanoliposomal formulation of irinotecan (nal-IRI; also known as MM-398 or PEP02; ref. 9). nal-IRI features very high drug loading efficiency, a high drug payload, and marked *in vivo* drug retention that

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also stabilizes the active lactone configuration of irinotecan. The pharmacokinetic properties of the encapsulated irinotecan were dramatically altered in the plasma of female rats, with a 344× increase in the area under the curve (AUC), an 8.5× decrease in the volume of distribution, and a 39.6× increase in the half-life of the total drug. Pharmacokinetic analysis in a clinical study confirmed these performance characteristics of nal-IRI in patients (10).

Plasma drug concentrations cannot readily be translated into therapeutic effect; a sufficient amount of active therapeutic agent must be transported to the tumor site of action (i.e., be available for uptake by cancer cells) to observe favorable drug activity (11). The transport of macromolecules across the tumor vasculature is a complex process depending on vessel perfusion, surface area, and permeability, as well as tumor and drug characteristics. Several studies have used mathematical models to understand liposomal drug delivery within solid tumors (12, 13). Of particular interest is work done by Hendriks and colleagues (14), where the authors constructed a computational model to describe the parameters that affect the tumor delivery of pegylated liposomal doxorubicin, the first liposomal anticancer agent to receive clinical approval. The study concluded that liposome pyruvate kinase (PK) and tumor permeability to liposomes (tumor deposition) were the most important parameters controlling liposomal drug delivery to tumors.

In the case of nal-IRI, the complex metabolism (15) and mechanism of action (8, 16) of free irinotecan, in addition to the above-mentioned parameters, may play a role in the overall liposomal irinotecan delivery within tumors. In this study, we describe a systems pharmacology approach to identify critical parameters that differentiate nal-IRI from free irinotecan with regard to *in vivo* activity. A mechanistic tumor PK model was developed and trained to describe CPT-11 and SN-38 levels observed in plasma and tumor, following administration of either nal-IRI or free irinotecan in tumor xenografts. A model sensitivity analysis was performed to identify the critical parameters driving *in vivo* activity, which were then experimentally confirmed by measuring these factors in multiple cell line and patient-derived xenograft models. The findings in this study highlight critical parameters that could serve as potential biomarkers to identify cancer indications and patient populations with an increased likelihood of nal-IRI responsiveness.

Materials and Methods

Materials and nal-IRI preparation

nal-IRI was prepared as previously described (9) using a lipid composition of DSPC, cholesterol, and PEG-DSPE (3:2:0.015, mol:mol:mol), an initial drug-to-lipid ratio of 500 g drug/mol phospholipid, and extrusion through 0.1 μm polycarbonate filters. The resulting preparations displayed a particle size of 111 nm (with polydispersity index of 0.04), and a drug load of 473 mg irinotecan-HCl/mmol phospholipid. All lipids were obtained from Avanti Polar Lipids Inc. Irinotecan hydrochloride was purchased from the pharmacy. Acetic acid, methanol, and acetonitrile were from EMD Chemicals Inc. Water and trifluoroacetic acid (TFA) were from J.T. Baker. Fetal bovine serum was from Tissue Culture Biologicals and phosphate-buffered saline (PBS) was purchased from Life Technologies.

Cell culture

Cell lines [HT-29 (colon), SK-ES-1 (Ewing sarcoma), A549 (lung), LoVo (colon), MDA-MB-231 (breast)] were obtained from the ATCC, whereas A2780 cells (ovarian) was obtained from Sigma-Aldrich. Cells from the ATCC and Sigma were received in 2010. All cells were authenticated before receipt and were propagated for less than 6 months after resuscitation. Cultures are regularly tested for *Mycoplasma*. All cell lines were cultured in humidified CO_2 atmosphere at 37°C using media recommended by the manufacturer.

Pharmacokinetic and tissue biodistribution study

Five-week-old female NOD/SCID mice were purchased from Charles River Laboratory. The care and treatment of experimental animals were in accordance with the Institutional Animal Care and Use Committee guidelines. Subcutaneous tumors were established by injecting 10 million HT-29 cells into the right flank of mice. When the average tumor volume reached approximately 200 mm^3, mice were randomized into groups ($n = 4$/time point) that received a single intravenous (i.v.) dose of nal-IRI at 5, 10, 20, or 40 mg/kg. Following 1, 4, 8, 24, 48, 72, and 168 hours after a single dose, mice were sacrificed and perfused with PBS before harvest of tumor and other normal tissues.

Antitumor activity studies

Five-week-old female NOD/SCID mice were purchased from Charles River Laboratory. Subcutaneous tumors were established by injecting 10 million HT-29 and SK-ES-1 cells or 5 million A549 cells into the right flank of mice. Tumor growth was measured twice per week by calipers and calculated with formula: $width^2 \times length \times 0.52$. When the average tumor volume reached approximately 200 mm^3, mice were randomized into treatment groups (n = 5–8/group) that received weekly i.v. dose of PBS (control), free irinotecan (50 mg/kg), or nal-IRI at various doses ranging from 1.25 to 20 mg/kg.

Tumor growth inhibition (TGI) was calculated with formula:

$$\mathrm{TGI}(\%) = \left[1 - \frac{(V_{\mathrm{treated}}(d_{\mathrm{final}}) - V_{\mathrm{treated}}(d_0))}{(V_{\mathrm{control}}(d_{\mathrm{final}}) - V_{\mathrm{control}}(d_0))}\right] \quad \text{(A)}$$

where V_{treated} and V_{control} represent the volumes of tumor at a given time point following treatment with drug or PBS, and d_0 and d_{final} represent first day and final day of treatment, respectively.

Characterizing tumors from cell-line and patient-derived xenografts

The cell line–derived xenografts (HT-29, SK-ES-1, A549, MDA-MB-231, LoVo, AsPC-1, and A2780) were established as described above. The patient-derived tumor models [CTG-0062 (colorectal), CTG-0079 (colorectal), CTG-0252 (ovarian), CTG-0288 (pancreatic), CTG-0158(lung), and CTG-0283 (pancreatic)] were established by Champions Oncology using their Champions TumorGraft (CTG) technology. When the

average tumor volume reached approximately 300 mm^3, mice were randomized into treatment groups ($n = 4$/group) that received single i.v. dose of either PBS or nal-IRI at 10 mg/kg. Before tumor collection, intracardial perfusion was performed to remove the blood components from the tumor compartment. Briefly, a butterfly needle (23G) connected to a 10-mL syringe filled with PBS is inserted into the left ventricle. Inferior vena cava is cut and animal is perfused with 10-mL PBS (within 1–2 minutes). The control tumors were harvested 24 hours after PBS administration and used for the irinotecan activation assay, whereas the treated tumors were harvested either 24 or 72 hours following nal-IRI treatment and used for high-performance liquid chromatography (HPLC) analysis.

HPLC quantification of CPT-11 and SN-38

Tumor and normal tissues were analyzed for CPT-11 and SN-38 concentrations using a modification of the method previously described (9). Briefly, tissues were weighed and homogenized for 2 minutes in 20% w/v water using a TissueLyser (Qiagen). The homogenates were extracted by mixing 0.1 mL homogenate with 0.9 mL 1% acetic acid/methanol followed by 10 seconds vortexing and placing at −80°C for 1 hour. The samples were centrifuged at 10,000 rpm for 10 minutes at room temperature and supernatants collected for HPLC analysis (Dionex). The samples and standards (CPT-11 and SN-38) were analyzed using a C18 reverse phase column (Synergi Polar-RP 80A 250 × 4.60 mm 4 μm column). The drug metabolites were eluted running a gradient from 30% acetonitrile; 70% 0.1% TFA/H_2O to 68% acetonitrile; 32% 0.1% TFA/H_2O during a 13 minutes span at a flow rate of 1.0 mL/min. The initial elute composition was restored after 14 minutes and continued for 6 minutes before the next injection. The CPT-11 peak was detected at approximately 7.7 minutes and the SN-38 peak eluted at approximately 8.4 minutes, using an in-line fluorescence detector excited at 372 nm and emitting at 556 nm.

Irinotecan activation assay

Tumor tissue lysates were prepared by homogenizing the tissue in 6% w/v 0.1 M Tris HCL/1% Triton X-100 solution (pH7.5) using a TissueLyser for 2 to 4 minutes. Protein concentration of lysates was measured using the BCA reagent (Thermo Scientific). Lysates (250 μg of protein) were mixed with an equal volume of 10 μmol/L irinotecan and incubated at 37°C. Following 24 hours of incubation the reaction was terminated by adding an equal volume of 1% acetic acid/methanol and samples centrifuged at 10,000 rpm for 15 minutes. The supernatant was processed for HPLC quantification of CPT-11 and SN-38 as described above.

Statistical analysis

The statistical significance of differences between groups was analyzed with the one-way ANOVA test. Results were considered statistically significant at $P < 0.05$. The analysis was performed using GraphPad Prism 6.01.

Model development and simulation

Pharmacokinetic profiles of metabolites in plasma and tumor from free irinotecan and nal-IRI were described by using multi-compartmental models (Fig. 1B). The model equations are explained and summarized in the Supplementary

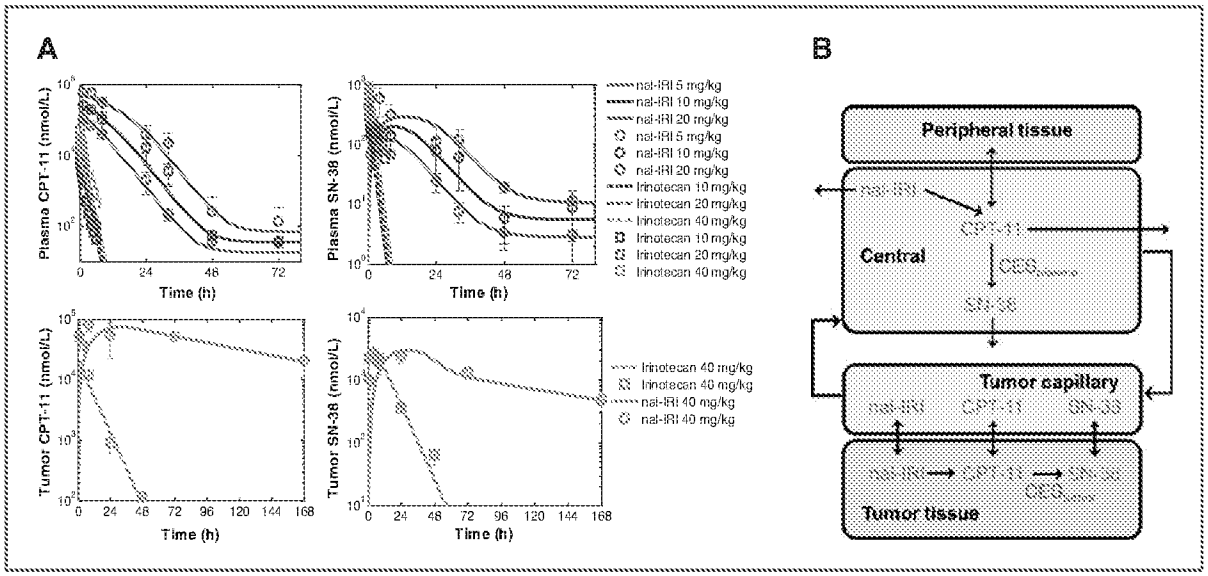

Figure 1. Pharmacokinetic profile of nal-IRI and free irinotecan. A, plasma and tumor PK of nal-IRI were compared with free irinotecan in HT-29 xenograft bearing mice. NOD-SCID mice bearing HT-29 tumors were treated with single i.v. dose of free irinotecan or nal-IRI. Plasma and tumors were collected at various intervals and the CPT-11 and SN-38 were measured by HPLC analysis ($n = 4$ animals/time point). Plasma PK data for free irinotecan were taken from Kaneda et al. (35). Solid lines, represent the model simulations for nal-IRI, whereas dashed lines represent the model simulations for free irinotecan. B, diagram of the mechanistic tumor pharmacokinetic model developed to describe the various steps in metabolism, pharmacokinetics and tumor deposition of nal-IRI.

Q13 Table 1. Summary of model parameters for plasma PK and tumor deposition models

Name	Value	Units	Description	Reference
Plasma PK model parameters				
For free irinotecan				
Cl_{CPT-11}	1.222e−4	L/min	Plasma clearance rate of CPT-11	Estimated
Cl_{SN-38}	2.138e−5	L/min	Plasma clearance rate of SN-38	Estimated
$k_{12,CPT-11}$	8.444e−5	1/min	Rate of CPT-11 transport from plasma to peripheral compartment	Estimated
$k_{21,CPT-11}$	4.213e−2	1/min	Rate of CPT-11 transport from peripheral to plasma compartment	Estimated
$k_{12,SN-38}$	2.656e−5	1/min	Rate of CPT-11 transport from plasma to peripheral compartment	Estimated
$k_{21,SN-38}$	3.44e−4	1/min	Rate of CPT-11 transport from peripheral to plasma compartment	Estimated
$V_{max,CES,p}$	2.263e−1	nmol/min	Maximum rate coefficient for CES enzyme in plasma compartment	Estimated
$K_{m,CES,p}$	2.67e5	nmol/L	Michaelis-Menten constant for CES enzyme in plasma compartment	Estimated
V_p	9.46e−5	L	Volume of plasma compartment	Estimated
V_{ph}	0.02	L	Volume of peripheral compartment	Fixed (14)
For nal-IRI				
$Cl_{nal-IRI}$	1.87e−7	L/min	Plasma clearance rate of nal-IRI	Estimated
Cl_{CPT-11}	1.634e−5	L/min	Plasma clearance rate of CPT-11	Estimated
Cl_{SN-38}	2.957e−6	L/min	Plasma clearance rate of SN-38	Estimated
$k_{12,CPT-11}$	1.619e−4	1/min	Rate of CPT-11 transport from plasma to peripheral compartment	Estimated
$k_{21,CPT-11}$	5.349e−7	1/min	Rate of CPT-11 transport from peripheral to plasma compartment	Estimated
$V_{max,Release,p}$	8.443e−6	nmol/min	Maximum rate coefficient for CPT-11 release from nal-IRI in plasma compartment	Estimated
$K_{m,Release,p}$	2.04	nmol/L	Michaelis-Menten constant for CPT-11 release from nal-IRI in plasma compartment	Estimated
$V_{max,CES,p}$	5.943e−2	nmol/min	Maximum rate coefficient for CES enzyme in plasma compartment	Estimated
$K_{m,CES,p}$	1.198e−5	nmol/L	Michaelis-Menten constant for CES enzyme in plasma compartment	Estimated
V_p	1.12e−3	L	Volume of plasma compartment	Estimated
V_{ph}	0.02	L	Volume of peripheral compartment	Fixed (14)
Tumor deposition model parameters				
Q_{tumor}	2.119e−6	L/min	Blood flow rate to tumor	(14)
$PS_{nal-IRI}$	7.858e−5	L/min/kg	Tissue permeability coefficient of nal-IRI	Estimated
PS_{CPT-11}	1.851e−3	L/min/kg	Tissue permeability coefficient of CPT-11	Estimated
PS_{SN-38}	2.687e−2	L/min/kg	Tissue permeability coefficient of SN-38	Estimated
$\sigma_{nal-IRI}$	3.181e−3		Tissue-capillary partition coefficient of nal-IRI	Estimated
σ_{CPT-11}	5.24e−1		Tissue-capillary partition coefficient of CPT-11	Estimated
σ_{SN-38}	2.109e−1		Tissue-capillary partition coefficient of SN-38	Estimated
$k_{Release,t}$	1.681e−4	1/min	Rate coefficient for +CPT-11 release from nal-IRI in tumor tissue compartment	Estimated
$V_{max,CES,t}$	2.17e−2	nmol/min	Maximum rate coefficient for CES enzyme in tumor tissue compartment	Estimated
$K_{m,CES,t}$	2.3e−6	nmol/L	Michaelis-Menten constant for CES enzyme in tumor tissue compartment	Estimated
V_{cap}	7e−7	L	Volume of tumor capillary compartment	(14)
V_t	1e−5	L	Volume of tumor tissue compartment	Fixed

Data. The models were built and implemented using Simbiology toolbox in MATLAB 8.2 (The MathWorks).

Results

nal-IRI displays a prolonged exposure in both plasma and tumor compared with free irinotecan

The pharmacokinetic profiles of the prodrug CPT-11 and its active metabolite SN-38 were measured in plasma and tumors following administration of either free irinotecan or nal-IRI (Fig. 1A). At similar doses of both free irinotecan and nal-IRI, the CPT-11 and SN-38 plasma levels cleared rapidly from circulation within 8 hours after free irinotecan injection, whereas the levels of CPT-11 and SN-38 following nal-IRI administration were persistent and remained in circulation for over 50 hours. An approximately 10-fold higher plasma CPT-11 peak level was observed with nal-IRI as compared with free irinotecan. However, the plasma peak level of SN-38 achieved with nal-IRI was 10-fold lower compared with free irinotecan, probably due to the ability of the lipid bilayer to protect the conversion of prodrug CPT-11 to SN-38 by the systemic CES enzyme present in mouse models (17). Administration of free irinotecan resulted in the clearance of greater than 90% of CPT-11 from tumors within 24 hours; however, following nal-IRI administration, CPT-11 levels persisted above

10,000 nmol/L levels for 168 hours. Similar peak levels of SN-38 were achieved with both free irinotecan and nal-IRI in HT-29 tumors, though a prolonged SN-38 exposure for up to 168 hours (measured as the AUC from 0 to 168 hours) was achieved with nal-IRI as compared with less than 48 hours tumor exposure with free irinotecan. In summary, CPT-11 and SN-38 were still present in tumors at 168 hours following nal-IRI administration, though both CPT-11 and SN-38 had cleared from plasma.

Tumor SN-38 duration drives *in vivo* activity

We developed a mechanistic PK model to identify the determinants that may differentiate the plasma and tumor PK profiles between free irinotecan and nal-IRI (Fig. 1B). The experimental PK data were used to estimate the optimal model parameters (Table 1) fitting the model simulations within the standard deviations of *in vivo* PK profiles of both CPT-11 and SN-38 (Fig. 1A). As the *in vitro* cytotoxic effects of irinotecan on tumor cells is dependent on the concentration and the time of exposure of cells to active metabolite SN-38 (7, 8), we sought to understand if the overall plasma and tumor SN-38 exposure predicts the *in vivo* activity of both nal-IRI and free irinotecan. The trained model determined that a 5-fold higher dose of free irinotecan (50 mg/kg) was required to achieve similar SN-38 exposure in both plasma and tumor as compared with nal-IRI (10 mg/kg; Fig. 2A). The TGI of HT-29 xenograft model at these equal exposure doses, was significantly greater with nal-IRI (~110%) treatment as compared with free irinotecan (~40%), despite the 5-fold lower total dose administered (*, $P < 0.05$,

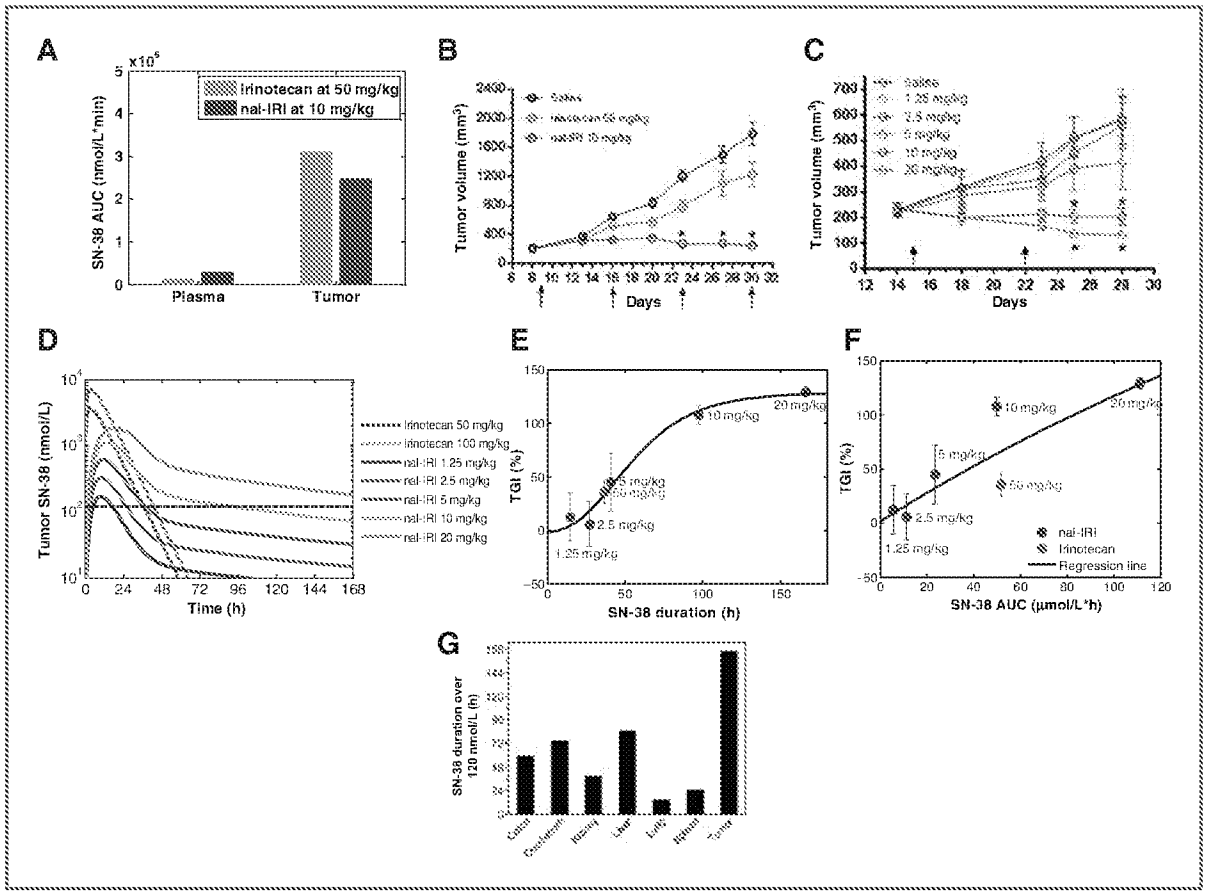

Figure 2. Relation of nal-IRI *in vivo* activity to tumor SN-38 duration. A, model predictions for similar SN-38 AUC in plasma and tumor following free irinotecan (50 mg/kg) and nal-IRI (10 mg/kg) administration. B, tumor response observed in HT-29 xenograft following weekly administration (arrows) of 50 mg/kg free irinotecan and 10 mg/kg nal-IRI ($n = 8$/group). The tumor volumes for nal-IRI (10 mg/kg) were significantly lower (*, $P < 0.05$) compared with saline and irinotecan groups (one-way ANOVA test). C, tumor response in HT-29 xenografts following weekly administration (arrows) of various nal-IRI doses ($n = 5$/group). The tumor volumes for nal-IRI (10 mg/kg) and nal-IRI (20 mg/kg) groups was significantly lower (*, $P < 0.05$) compared with saline tumors on Day 25 and Day 28 (one-way ANOVA test). D, model simulations were used to compare tumor SN-38 concentration following the administration of varying doses of free irinotecan or nal-IRI. Black dashed line represents threshold concentration of 120 nmol/L to determine tumor SN-38 duration. E, TGI(%) achieved by nal-IRI and free irinotecan treatment in HT-29 xenografts were compared with the tumor SN-38 duration above 120 nmol/L (E) and SN-38 AUC (F) at varying doses of nal-IRI or free irinotecan Solid lines represent nonlinear regression lines based on five parameter logistic curve fitting. G, the SN-38 duration over a threshold of 120 nmol/L was computed from the pharmacokinetic profiles of SN-38 in tumor and normal tissues following 20 mg/kg of nal-IRI.

one-way ANOVA test; Fig. 2B). In addition, other studies have shown no additional HT-29 TGI at the maximum tolerated dose of free irinotecan (100 mg/kg; ref. 18). To identify a dose level of nal-IRI that gave comparable *in vivo* activity to 50 mg/kg free irinotecan, we performed a dose escalation study in the HT-29 xenograft model (Fig. 2C). nal-IRI at 5 mg/kg showed partial inhibition of tumor growth (~40% TGI) that was comparable with 50 mg/kg free irinotecan, whereas 10 mg/kg and 20 mg/kg nal-IRI showed significant (*, $P < 0.05$, one-way ANOVA test) TGI compared with saline (~110%–130% TGI). Furthermore, we have previously tested control liposomes (that have comparable composition with nal-IRI except for the absence of irinotecan, the active pharmaceutical ingredient) and did not observe any TGI (data not shown).

The intratumor SN-38 concentrations achieved from 50 to 100 mg/kg doses of free irinotecan and 1.25, 2.5, 5, 10, and 20 mg/kg doses of nal-IRI were then simulated using the trained mechanistic PK model (Fig. 2D). Although a nal-IRI dose of 5 mg/kg achieved similar TGI as 50 mg/kg free irinotecan, the tumor SN-38 AUC and peak levels were approximately 2-fold and 6-fold lower respectively for nal-IRI as compared with free irinotecan. Furthermore, we noted at these doses both drugs were able to maintain the tumor SN-38 concentration above 120 nmol/L for the same duration of approximately 40 hours. To determine if the tumor SN-38 concentration impacts *in vivo* activity, we used the tumor SN-38 concentration of 120 nmol/L as a threshold. We also determined the duration for which the various doses of nal-IRI or free irinotecan could maintain the tumor SN-38 concentration above 120 nmol/L, hereon referred to as "tumor SN-38 duration." A sigmoidal relationship between TGI (%) and tumor SN-38 duration (Fig. 2E) was observed for both nal-IRI and free irinotecan ($R^2 = 0.62$). However, when comparing TGI (%)with tumor SN-38 AUC (Fig. 2F) the relationship was less significant ($R^2 = 0.45$), due to the lower TGI (%) achieved by 50 mg/kg free irinotecan compared with 10 mg/kg nal-IRI. We also observed longer SN-38 duration in tumors (>100 hours) compared with normal tissues (<72 hours; Fig. 2G and Supplementary Fig. S1).

Identification of liposome tumor permeability and local tumor activation as critical determinants for tumor SN-38 duration

A local sensitivity analysis on the model parameters was performed to identify processes impacting the tumor SN-38 duration (Supplementary Data). In response to the administration of free irinotecan (50 mg/kg), the tumor SN-38 duration was relatively insensitive to most model parameters (Fig. 3A), suggesting the inability of free irinotecan to modulate it. In contrast, several model parameters were found to significantly impact tumor SN-38 duration following the administration of nal-IRI (10 mg/kg; Fig. 3B). The sensitive parameters for nal-IRI can be classified into three different categories: (i) PK, rate of breakdown of liposomes in blood (Release rate in blood, $V_{max,Release,p}$), (ii) activation of prodrug CPT-11 to SN-38 by CES enzyme (CES activity in tumor; $V_{max,CES,t}$ and blood; $V_{max,CES,p}$), and (iii) liposome uptake within tumors, that is, nal-IRI tumor deposition (nal-IRI tumor permeability, $PS_{nal\text{-}IRI}$). Among these parameters, the release rate in plasma negatively affected tumor SN-38 duration due to a decrease in the overall systemic exposure of nal-IRI. CES enzyme activity, particularly from tumor CES (local tumor activation of irinotecan) and nal-IRI permeability (tumor deposition), positively affected the tumor SN-38 duration. To assess the identifiability of parameter estimates, log likelihood profiling was performed for the sensitive parameters, $V_{max,CES,t}$ and $PS_{nal\text{-}IRI}$ (19). The confidence intervals suggested that both parameters were precisely estimated (Supplementary Fig. S2).

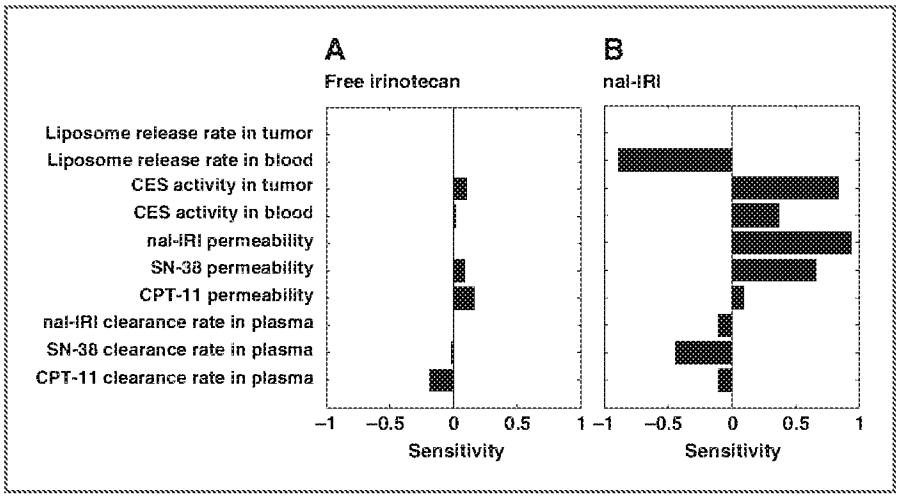

Figure 3. Model parameters impacting tumor SN-38 duration. Sensitive analyses for free irinotecan (A) and nal-IRI (B) were performed on key model parameters that are responsible for plasma clearance, tissue deposition and metabolic reactions. Parameters whose values were not estimated in this study including compartment volumes and tumor blood flow were excluded from the analysis. The doses of free irinotecan (50 mg/kg) and nal-IRI (10 mg/kg) that achieved similar SN-38 plasma and tumor exposure were used for sensitivity analysis. The model parameters were modulated by 10% and their effect on tumor SN-38 duration was determined as a sensitivity index (Supplementary Equation S6).

Biologic variability and simulated perturbation of nal-IRI tumor deposition and local activation

To determine the biologic relevance of these sensitive parameters toward driving tumor SN-38 duration (namely nal-IRI tumor deposition and local tumor activation of irinotecan), the parameters were measured in a panel of 13 xenograft models. We used the total CPT-11 concentrations in tumors as a surrogate for nal-IRI tumor deposition as model simulations based on nal-IRI pharmacokinetics showed that majority of CPT-11 in plasma and tumor was encapsulated and protected within the liposomes and less than 10% was available as free CPT-11 (Supplementary Fig. S3). The intra tumor concentrations of CPT-11 varied substantially across the tumor panel (Fig. 4A). The tumor models from cell-lines displayed overall higher levels of prodrug CPT-11 deposition (from 5,000–15,000 ng/g) as compared with patient-derived tumor models (1,000–2,000 ng/g). In addition, a high degree of variability was observed between individual tumors within the same xenograft model (66% average coefficient of variation). Model simulations were used to test the effect of altering nal-IRI tumor deposition on tumor SN-38 duration (Fig. 4B). By decreasing the nal-IRI permeability parameter to zero, which simulates an impermeable tumor microenvironment, the tumor SN-38 duration of approximately 100 hours achieved

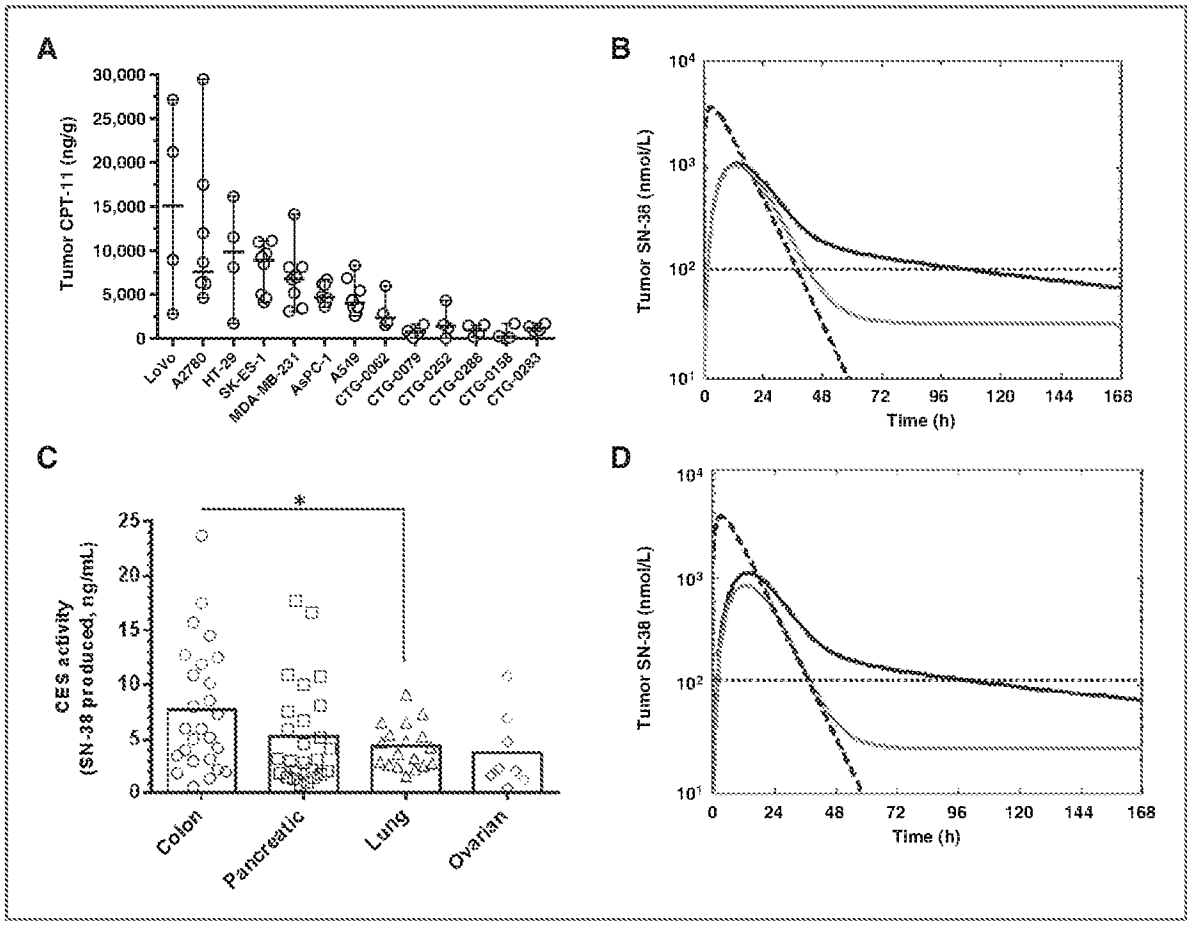

Figure 4. *In vivo* variability in nal-IRI tumor deposition and local activation. A, intratumor CPT-11 concentrations were measured across cell-line derived (HT-29, SK-ES-1, A549, MDA-MB-231, LoVo, AsPC-1, and A2780) and patient-derived (CTG-0062, CTG-0079, CTG-0252, CTG-0288, CTG-0158, and CTG-0283) tumor models. Tumor bearing mice were administered a single i.v. dose of 10 mg/kg nal-IRI and tumors excised 24 hours later. CPT-11 concentrations were determined in the tumor lysates using HPLC analysis as described in methods ($n = 4$–8 tumors/model). B, the effect of nal-IRI permeability on tumor SN-38 concentrations was simulated by reducing the nal-IRI permeability parameter, $PS_{nal\text{-}IRI}$ to zero. The simulated tumor SN-38 concentrations were compared at the equal exposure doses of 10 mg/kg nal-IRI and 50 mg/kg free irinotecan. Black solid line: nal-IRI (10 mg/kg) with base $PS_{nal\text{-}IRI}$. Gray solid line: nal-IRI (10 mg/kg) with zero $PS_{nal\text{-}IRI}$. Black dashed line: free irinotecan (50 mg/kg). Dotted line, threshold concentration of 120 nmol/L. (C) CES activity for 80 patient-derived xenograft tumors across different indications was determined using *ex vivo* irinotecan activation assay. Tumor lysates (250 μg of protein) from untreated mice was incubated with free irinotecan (5 μmol/L) for 24 hours at 37°C and the amount of SN-38 produced was measured with HPLC analysis (*, $P < 0.05$; *t* test). D, the effect of knocking out tumor CES activity on tumor SN-38 duration was simulated by reducing the tumor CES parameter, $V_{max,CES,t}$ to zero. The simulated tumor SN-38 concentrations were compared at the equal exposure doses of 10 mg/kg nal-IRI and 50 mg/kg free irinotecan. Black solid line: nal-IRI 10 mg/kg with base $V_{max,CES,t}$. Gray solid lines: nal-IRI 10 mg/kg with zero $V_{max,CES,t}$. Black dashed line: free irinotecan 50 mg/kg with base $V_{max,CES,t}$. Dotted line, threshold concentration of 120 nmol/L.

with 10 mg/kg nal-IRI was substantially reduced to approximately 50 hours and approached the levels observed with 50 mg/kg free irinotecan. Taken together, these results suggest that the tumor deposition of nal-IRI is highly tumor specific and will dramatically impact tumor SN-38 duration.

To determine the degree to which local tumor activation of irinotecan varied in human tumors, we measured CES activity using an *ex vivo* assay in a panel of 80 patient-derived tumors. The tumor lysates varied in their ability to activate prodrug irinotecan and produce SN-38 (1–25 ng/mL SN-38 produced), suggesting a high degree of variability in local tumor activation of irinotecan across indications. A significant difference in local tumor activation of irinotecan was observed between colon and lung tumors ($P < 0.05$). However, there was no significant difference between other indications, which may be due to high variability observed within each indication (Fig. 4C). The impact of varying the tumor CES activity on tumor SN-38 duration was evaluated by simulating a knockout of tumor CES enzyme (Fig. 4D). In the absence of local tumor activation, tumor SN-38 duration with nal-IRI (10 mg/kg) decreased from approximately 100 to 40 hours, similar to that achieved by free irinotecan (50 mg/kg).

nal-IRI tumor deposition and local activation collectively predict tumor SN-38 duration

The relative contribution of nal-IRI tumor deposition and local tumor activation on tumor SN-38 duration was evaluated using model simulations. On the basis of the findings from the sensitivity analysis (Fig. 3B), nal-IRI permeability ($PS_{nal\text{-}IRI}$) and tumor CES activity ($V_{max,CES,t}$) values were used to create a map relating these parameters to tumor SN-38 duration following nal-IRI administration (Fig. 5A). Model simulations predicted a concave relationship, where the tumor SN-38 duration is dependent upon both the tumor permeability and the tumor CES activity. The tumor SN-38 duration could be increased by either increasing the $PS_{nal\text{-}IRI}$ or $V_{max,CES,t}$ (white arrows) and the maximum tumor SN-38 duration of 168 hours was only reached with CES activity at 0.025 nmol/min and tumor permeability at 1.5E−4 L/min/kg.

To experimentally test the model predictions, we used the same panel of 13 xenograft models to measure the tumor concentrations of CPT-11 (as a surrogate for tumor deposition, Supplementary Fig. S4A); tumor SN-38 concentrations (as a surrogate for tumor SN-38 duration; Supplementary Fig. S4B) and CES activity (for local tumor activation of irinotecan). The experimental data supported the model simulations, confirming that the SN-38 concentration within tumors was dependent on both the tumor CPT-11 concentration and tumor CES activity (Fig 5B). All tumor models with high CPT-11 concentration >2,000 ng/g or high CES activity > 5 ng/mL displayed high tumor SN-38 concentrations ("red") ranging from 25 to 125 ng/mL (Supplementary Table S2). In certain tumor models, one of the parameters contributed predominantly toward higher SN-38 concentrations (black arrows). A2780 and SK-ES-1 tumors displayed high tumor SN-38 concentrations of 97 ng/mL and 127 ng/mL respectively (Supplementary Table S2), which was mainly due to high CPT-11 concentrations (>2,000 ng/g), whereas in other tumor models (CTG-0062 and AsPC-1) the CES activity (>5 ng/mL) was the dominant factor contributing toward high tumor SN-38 concentrations. Further tumor models with the lowest tumor SN-38 concentrations ranging from 5 to 12 ng/mL ("blue"), including several patient-derived tumor models (boxed area) also displayed lower tumor CPT-11 concentrations (<2,000 ng/g) and CES enzyme activity (<5 ng/mL).

Tumor SN-38 duration correlates with nal-IRI *in vivo* activity

In vivo tumor response studies were performed in three tumor models in which different tumor SN-38 durations had been observed (as indicated by tumor SN-38 concentration at 72 hours) to determine the impact of tumor SN-38 duration on *in vivo* activity of nal-IRI. The tumor volumes observed for both HT-29 (Fig. 6A) and SK-ES-1 (Fig. 6B) models were significantly

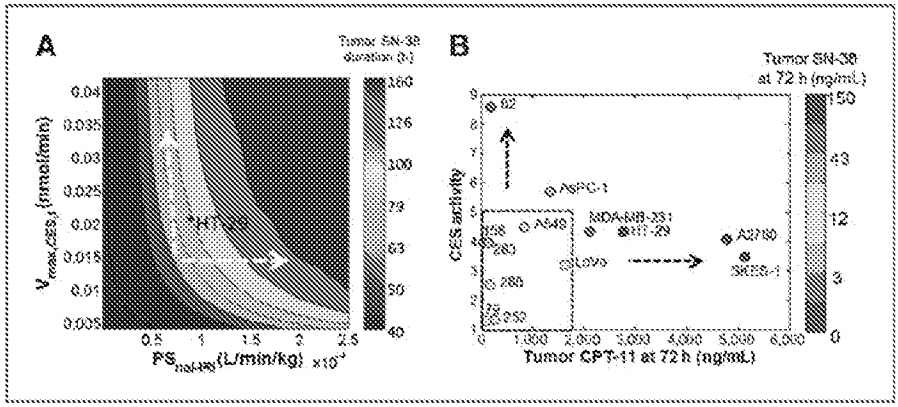

Figure 5. nal-IRI tumor deposition and local activation impacts tumor SN-38 duration. A, the effect of changing tumor CES activity and nal-IRI permeability parameters (arrows) on tumor SN-38 duration (color-coded in hours) in tumors was simulated. The optimal parameter values for HT-29 were marked with the symbol "*". B, experimental data in tumor xenograft models showing the impact of tumor CPT-11 and CES activity on tumor SN-38 concentrations. Tumor CES activity (as surrogate for local tumor activation of irinotecan) and tumor CPT-11 concentration at 72 hours (as surrogate for tumor deposition) for different xenograft models were plotted and color-coded on the basis of their SN-38 concentrations in the tumor 72 hours after nal-IRI (each data point represents median of $n = 4$–8 tumors). Dotted arrows, dependence of tumor SN-38 concentrations on tumor CPT-11 concentration and CES activity.

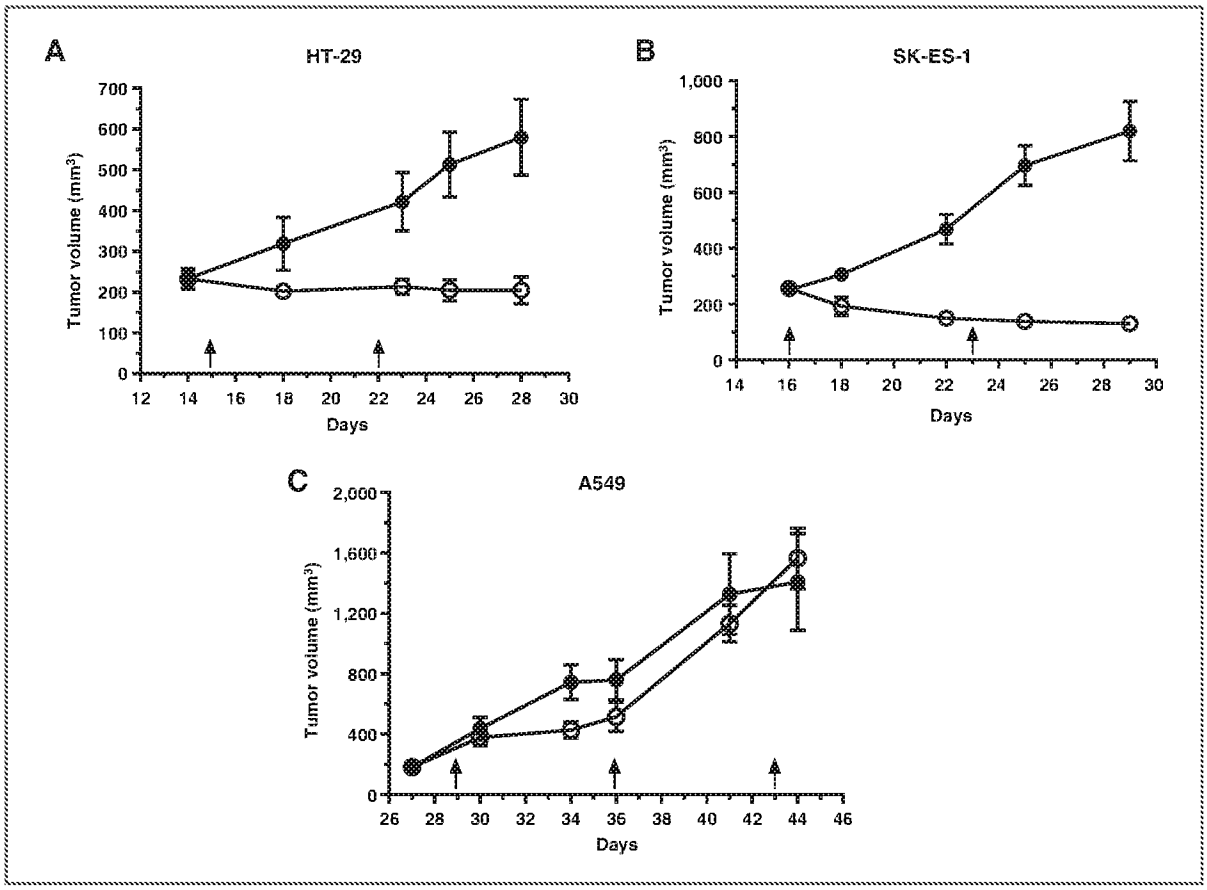

Figure 5. *In vivo* tumor response for nal-IRI. NOD SCID mice were inoculated with HT-29; colon (A), SK-ES-1; ewings (B) and A549; lung (C) cell lines. Tumor bearing mice were randomized when the tumor volume was approximately 200 mm^3. Each group received weekly i.v. dose (arrows) of either saline (●) or 10 mg/kg dose of nal-IRI (○). Tumor volumes were measured twice per week (n = 5–10 animals/group).

lower ($P < 0.05$) following 10 mg/kg nal-IRI as compared with untreated tumors. In both these models, tumor regression was observed immediately after the first dose and was sustained through the course of the study. A549 tumors achieved lower SN-38 tumor levels (Fig. 4A) and did not respond to nal-IRI treatment (Fig. 6C). Interestingly, both A549 and HT-29 cells displayed similar *in vitro* sensitivity to SN-38 with IC_{50} values of 53 and 44 nmol/L, respectively (20). In summary, nal-IRI induced stronger responses (~100%TGI) in tumor models that had higher tumor SN-38 duration (>~100 hours).

Discussion

The nal-IRI formulation dramatically alters the pharmacologic properties of irinotecan as well as its active metabolite, SN-38 (9). In this study, we identified a pharmacologic parameter—namely, tumor SN-38 duration—as a driver of irinotecan-based *in vivo* activity and propose biomarkers that can impact tumor SN-38 duration achieved by nal-IRI. Our study indicates that nal-IRI can completely inhibit tumor growth compared with free irinotecan, despite administering doses that achieve similar SN-38 exposure (measured as the AUC). Instead, the duration of prolonged exposure of SN-38 within tumors achieved by nal-IRI was shown to be a major pharmacologic determinant for *in vivo* activity in mice.

Several studies have shown improved *in vitro* cytotoxic activity of SN-38 when cells are exposed to drug for longer duration (21). The *in vitro* cell doubling time for HT-29 cells is approximately 20 hours (21), whereas *in vivo* the tumor volume doubles (Fig. 2B) at a slower rate (~8–9 days). In addition, at a given time only 35 to 50% of cells are in the S-phase of cell cycle wherein the maximum cytotoxicity of free irinotecan has been observed (21). Thus, to exert maximum cytotoxic effects across different cell-cycle phases, the cells have to be exposed to free irinotecan across multiple cell cycles. Our *in vivo* study confirms these findings as the free irinotecan is rapidly cleared from plasma and tumor tissue (tumor SN-38 duration of approximately 40 hours), thereby not allowing sufficient time for tumor cells to be exposed to SN-38 (for only 2 cell-cycle doubling time) as compared with more than 5 cell-cycle doubling times with nal-IRI (tumor SN-38 duration for >100 hours). Thus the extended exposure of tumor cells to SN-38, which is achieved by nal-IRI, can contribute toward the enhanced cytotoxicity as compared with free irinotecan.

We observed higher tumor concentrations of CPT-11 and SN-38 at 168 hours following administration of nal-IRI. In contrast, the peak plasma concentrations of SN-38 was lower with nal-IRI as compared with free irinotecan, suggesting that most of the CPT-11 from nal-IRI remains inside the liposomes and is protected from systemic conversion as described with free irinotecan (17). In addition, prolonged SN-38 duration from nal-IRI administration was observed only in tumors and much less in normal tissues, suggesting that toxicity might not be exacerbated by nal-IRI treatment. The preferentially accumulation of nal-IRI in tumors as compared with normal tissues can be attributed to the EPR effect, where the leaky vasculature in tumor facilitates the extravasation of liposomal nanoparticles and the defective lymphatic drainage helps increase the retention within tumor (1, 2). Thus, with the EPR effect, nal-IRI creates a large depot of CPT-11 only in tumors thereby prolonging tumor SN-38 duration. In contrast, free irinotecan can easily be transported in and out of the tissues with a short plasma half-life, resulting in minimal SN-38 duration in tumors.

The enhanced *in vivo* activity of nal-IRI as compared with free irinotecan was attributed to the ability of nal-IRI to extend the tumor SN-38 duration. Sensitivity analysis identified two key determinants that impact the ability of nal-IRI to extend tumor SN-38 duration—(i) nal-IRI tumor deposition, as measured by the extent of prodrug CPT-11 deposition within tumors and (ii) nal-IRI local activation, from prodrug CPT-11 to SN-38 facilitated by the local tumor CES enzyme. The experimental data, in this study supported the importance of each of these determinants. We observed high degree of variability in the overall nal-IRI tumor deposition across the 13 xenograft models that were tested. Several studies have highlighted a role for tumor permeability, tumor perfusion, and stromal matrix in limiting the delivery of therapeutic agents into tumors (22). In our model simulations, when the nal-IRI tumor permeability was decreased to zero, the benefit of higher tumor SN-38 duration with nal-IRI was negatively impacted and reduced to levels simulated for free irinotecan. We also observed that the tumors with lower nal-IRI deposition had considerable lower SN-38 tumor levels. These data are consistent with other findings suggesting that a dense tumor stroma can impede drug permeability and limit drug delivery within tumors (23, 24).

Use of tumor CES activity as a cellular parameter for predicting free irinotecan response had limited success both in preclinical (25, 26) and clinical studies.(27). Through the sensitivity analysis performed in this study, we identified CES activity as a critical parameter for nal-IRI activity. Tumor models that displayed high ability to activate CPT-11, achieved high tumor SN-38 concentrations despite limited deposition of CPT-11, thus suggesting the importance of local tumor CES enzyme expression in facilitating longer SN-38 exposure following nal-IRI administration. In fact, others have shown that *in vitro* and *in vivo* activity of free irinotecan can be enhanced by overexpressing of CES enzyme in tumor cells (28, 29). In addition to tumor cells expressing CES enzyme (30), other components of the intracellular matrix such as tumor-associated macrophages (TAMs) express CES1 enzyme and play a role in CPT-11 activation (31). In fact, we performed *in vitro* studies that confirmed the ability of TAMs to hydrolyze CPT-11 to SN-38 (Supplementary Fig. S5). Thus our data suggest, the extended tumor PK achieved by nal-IRI provides high local depot of prodrug CPT-11 for prolonged time thus allowing for activation by tumor CES enzymes. Collectively our data provide rational for investigating tumor CES enzyme activity as potential marker for nal-IRI activity.

Pharmacogenetic and pharmacodynamics markers such as Topo1 have shown limited correlations with free irinotecan response (6, 32–34). In addition to the intrinsic sensitivity of tumor cells to SN-38, our data indicate that the duration for which tumor cells are exposed to SN-38 (tumor SN-38 duration) also plays a critical role in driving treatment response to irinotecan. Tumor models with extended SN-38 duration (HT-29, SK-ES-1) showed robust *in vivo* response to nal-IRI, whereas A549 with shorter tumor SN-38 duration did not respond to therapy. The fact that *in vitro* sensitivity of both HT-29 and A549 to SN-38 is very similar (20) corroborates the finding that the duration of SN-38 is driving the tumor response.

In conclusion, our data demonstrate that nal-IRI enhances the pharmacokinetic profile of tumor SN-38, prolonging tumor exposure to SN-38 compared with free irinotecan, and therefore has the potential for therapeutic effect in human cancers. Liposome permeability and CES activity were the critical factors that emerged from model simulation of tumor SN-38 duration, which were experimentally shown to vary across and within tumor indications. Thus, translational research exploring the utility of tumor liposome permeability and local activation of irinotecan as biomarkers for nal-IRI clinical activity is warranted.

Disclosure of Potential Conflicts of Interest

D.C. Drummond and J.B. Fitzgerald have ownership interest (including patents) in Merrimack Pharmaceuticals, Inc. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: A.V. Kalra, J. Kim, S.G. Klinz, D.C. Drummond, U.B. Nielsen, J.B. Fitzgerald

Development of methodology: A.V. Kalra, J. Kim, S.G. Klinz

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.V. Kalra, N. Paz, J. Cain

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.V. Kalra, J. Kim, D.C. Drummond, J.B. Fitzgerald

Writing, review, and/or revision of the manuscript: A.V. Kalra, J. Kim, S.G. Klinz, D.C. Drummond, U.B. Nielsen, J.B. Fitzgerald

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Kim, N. Paz

Study supervision: A.V. Kalra, J.B. Fitzgerald

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EFS ID:	35141821
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Confirmation Number:	5137
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First Named Inventor/Applicant Name:	Eliel Bayever
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Inventors: Eliel BAYEVER, et al. Application No.: 15/809,815 Filed: November 10, 2017 Title: METHODS FOR TREATING METASTATIC PANCREATIC CANCER USING COMBINATION THERAPIES COMPRISING LIPOSOMAL IRINOTECAN AND OXALIPLATIN	Group Art Unit: 1612 Examiner: Celeste A. RONEY Confirmation No.: 5137

INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. § 1.97(b)

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Commissioner:

Pursuant to 37 C.F.R. §§ 1.56 and 1.97(b), Applicant brings to the attention of the Examiner the documents listed on the enclosed PTO/SB/08s. This Information Disclosure Statement is being filed before the mailing of a first Office action after the filing of a Request for Continued Examination under § 1.114 on February 11, 2019.

Copies of the listed foreign patent documents and non-patent literature documents are enclosed.

Applicant wishes to bring to the Office's attention Applicant's applications relating to liposomal irinotecan that are listed in the table below. Office Actions from these applications are listed in the accompanying PTO/SB/08s and copies are enclosed.

Application No.	Filing Date	Patent No.	Status	Examiner
11/121,294	5/2/2005	8,147,867	Issued	Shomer, Isaac
11/601,451	11/17/2006	8,658,203	Issued	Kishmore, Gollamudi S.
13/416,204	3/9/2012	8,329,213	Issued	Shomer, Isaac
13/654,373	10/17/2012	8,703,181	Issued	Shomer, Isaac
14/151,632	1/9/2014		Abandoned	Kishmore, Gollamudi S.
14/175,365	2/7/2014	8,992,970	Issued	Shomer, Isaac
14/632,422	2/26/2015	9,717,723	Issued	Shomer, Isaac
14/879,302	10/9/2015	9,730,891	Issued	Shomer, Isaac
14/879,358	10/9/2015		Abandoned	Shomer, Isaac
14/964,239	12/9/2015		Abandoned	Shomer, Isaac
14/965,140	12/10/2015	9,724,303	Issued	Shomer, Isaac
14/966,458	12/11/2015	9,782,349	Issued	Shomer, Isaac
14/979,666	12/28/2015		Abandoned	Shomer, Isaac
15/227,561	8/3/2016		Published	Shomer, Isaac
15/227,631	8/3/2016		Published	Shomer, Isaac
15/213,127	7/18/2016		Abandoned	
15/296,536	10/18/2016	9,737,528	Issued	Kishmore, Gollamudi S.
15/363,761	11/29/2016		Published	Roney, Celeste A.
15/363,923	11/29/2016		Abandoned	Roney, Celeste A.
15/363,978	11/29/2016		Published	Roney, Celeste A.
15/364,021	11/29/2016		Abandoned	Liu, Tracy
15/664,976	7/31/2017		Published	Shomer, Isaac
15/896,389	2/14/2018		Published	Shomer, Isaac
15/896,436	2/14/2018		Published	Shomer, Isaac
14/406,776	12/10/2014	9,452,162	Issued	Strong, Tori
14/812,950	7/29/2015	9,339,497	Issued	Strong, Tori
14/844,500	9/3/2015	9,364,473	Issued	Strong, Tori
14/851,111	9/11/2015	9,492,442	Issued	Strong, Tori
15/059,640	3/3/2016		Abandoned	Strong, Tori
15/241,128	8/19/2016	9,717,724	Issued	Strong, Tori
15/341,377	11/2/2016		Abandoned	Strong, Tori
15/341,619	11/2/2016		Abandoned	Strong, Tori
15/652,513	7/18/2017		Abandoned	Strong, Tori
15/664,930	7/31/2017		Abandoned	Strong, Tori
16/012,351	6/19/2018		Pending	TBD
16/012,372	6/19/2018		Pending	TBD
14/964,571	12/9/2015		Published	Baek, Bong-Sook
15/375,039	12/9/2016		Abandoned	Baek, Bong-Sook
15/928,649	3/22/2018		Abandoned	
16/036,885	7/16/2018		Pending	TBD
15/337,274	10/28/2016	9,895,365	Issued	Packard, Benjamin J.

Application No.	Filing Date	Patent No.	Status	Examiner
15/852,551	12/22/2017		Published	Packard, Benjamin J.
15/241,106	8/19/2016		Abandoned	Roney, Celeste A.
15/809,815	11/10/2017		Published	Roney, Celeste A.
15/403,441	1/11/2017		Abandoned	Packard, Benjamin J.
15/331,648	10/21/2016		Abandoned	Shomer, Isaac
15/331,393	10/21/2016		Abandoned	Shomer, Isaac
15/331,318	10/21/2016		Abandoned	Shomer, Isaac
15/645,645	7/10/2017		Abandoned	Shomer, Isaac
15/655,592	7/20/2017		Abandoned	
15/661,868	7/27/2017		Abandoned	Shomer, Isaac
15/908,443	2/28/2018		Abandoned	
15/768,352	4/13/2018		Pending	Shomer, Isaac
15/967,633	5/1/2018		Abandoned	
15/967,638	5/1/2018		Pending	Shomer, Isaac
15/598,633	5/18/2017		Abandoned	Ricci, Craig D.
15/948,571	4/9/2018		Abandoned	

Applicant respectfully requests that the Examiner consider the listed documents and indicate that they were considered by making appropriate notations on the attached form.

This submission does not represent that a search has been made or that no better art exists and does not constitute an admission that each or all of the listed documents are material or constitute "prior art." If the Examiner applies any of the documents as prior art against any claim in the application and Applicant determines that the cited documents do not constitute "prior art" under United States law, Applicant reserves the right to present to the U.S. Patent and Trademark Office the relevant facts and law regarding the appropriate status of such documents.

Applicant further reserves the right to take appropriate action to establish the patentability of the claimed invention over the listed documents, should one or more of the documents be applied against the claims of the present application.

Please grant any extensions of time required to enter this response and charge any additional required fees to Deposit Account 506488.

Respectfully submitted,

McNeill Baur PLLC

Dated: February 13, 2019

By: /Mary R. Henninger, PhD/
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Sustained intratumoral activation of MM-398 results in superior activity over irinotecan demonstrated by using a systems pharmacology approach

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ABSTRACT

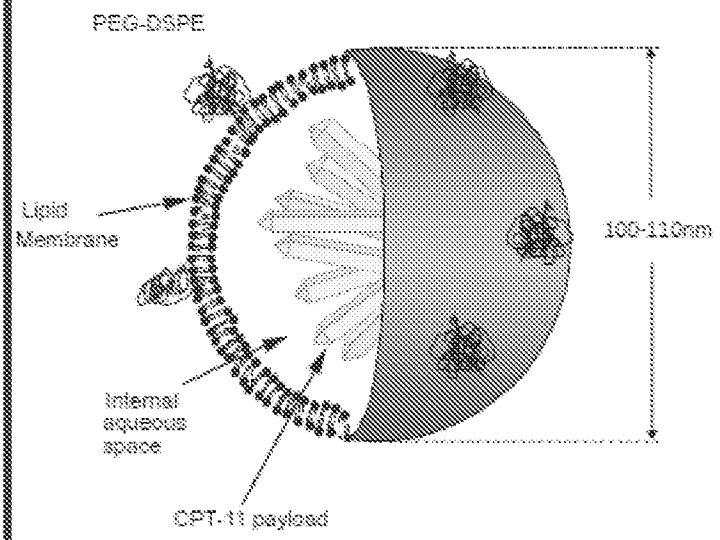
MM-398 is a stable nanotherapeutic encapsulation of the pro-drug irinotecan with an extended plasma half-life and higher intratumoral deposition compared with free-irinotecan. MM-398 is currently in multiple clinical trials, including a phase 3 trial for patients with advanced gemcitabine-resistant pancreatic cancer (NAPOLI-1). Pancreatic cancer has been described as being notoriously difficult to treat, potentially due to inadequate drug penetration through the dense stroma, or because the hypoxic tumor micro-environment suppresses cytotoxic activity. We sought to better understand how MM-398, a relatively large (100nm) liposomal nanotherapeutic, could potentially treat pancreatic cancer by determining the relative roles of systemic vs. local tumor activation of irinotecan in contributing to the activity of MM-398.

Using a systems pharmacology approach, we developed a mechanistic pharmacokinetic (PK) model of MM-398 and free-irinotecan to predict both plasma and intratumoral levels of irinotecan and SN-38. The model was trained with PK and biodistribution data from mice bearing HT-29 xenografts, which were administered intravenously with varying doses of MM-398 or free-irinotecan. Model simulations predicted that MM-398 resulted in equivalent SN-38 exposure (area under curve, AUC) in tumor at a five-fold lower dose than free-irinotecan. However, an *in vivo* animal activity study showed that 15-fold lower dose of MM-398 was sufficient to yield equal growth inhibition of HT-29 xenografts, which reveals the limit of relating simple AUC based exposure to *in vivo* tumor response. While intratumoral SN-38 exposure from free-irinotecan was limited to the first 48 hours after dosing, MM-398 maintained high levels of SN-38 throughout the week long time window. Further analysis of the exposure-response identified that the duration of intratumoral SN-38 levels above the threshold was a valid predictive marker for xenograft tumor response.

Identifying the source of intratumoral SN38 is confounded by the fact that the mouse species has an additional carboxylesterase (CES) that can convert irinotecan to SN-38 in serum. The serum SN-38/irinotecan ratio in mice is ten-fold higher than that observed in humans. In order to translate this preclinical observation into the clinic, it is critical to identify the role of mouse-specific serum CES on intratumoral SN-38 exposure. Thus, we performed a PK study with knockout mice lacking the Ces1c gene, which encodes serum CES, and then retrained our mechanistic PK model. Serum SN-38 levels in the Ces1c knockout mice were measurably decreased by ~85% in the central compartment. In contrast, simulating the effect of knock-out of either serum CES or tumor CES, predicts that the duration of intratumoral residence of SN-38 is significantly affected by tumor CES, rather than serum CES. This suggests that local activation to SN-38 by tumor CES as the main driver for SN-38 tumor residence, which in turn drives response.

In summary, we applied a systems pharmacology approach to identify the importance of tumor CES (local SN-38 generation) as one of the determinants of MM-398 response. Liposomal encapsulation of irinotecan dramatically alters the pharmacokinetic profile of SN-38 in the tumor, as well as tumor response, by maintaining SN-38 levels above the response -threshold. Local, sustained activity of this active irinotecan metabolite could result in prolonged cytotoxic and tumor-microenvironment modifications with beneficial effects on treatment of pancreatic cancer and other solid tumors.

MM-398 Background



Low-Pegylated

- Extended half-life with "passive" accumulation in tumor to achieve high drug levels
- 0.5 %mol PEG-DSPE levels

Non-Targeted

- Drug release in tumor into interstitial space or intracellular after endocytosis

Liposomal

- Effective packaging of CPT-11 with ~70,000 molecules/liposome

CPT-11

- CPT-11 prodrug converted to SN-38 by carboxylesterases
- SN-38 potency~>1000 CPT-11
- After conversion to SN-38 binds to TOPO1
- Induces single-strand breaks in DNA

Current Clinical Trials

- Ongoing Phase 3 monotherapy in 2nd line pancreatic cancer patients (NAPOLI-1)
- Ongoing Phase 2 combination with 5-FU & LV in 2nd line colorectal cancer

Systems Pharmacology Approach to Identify MOA for MM-398

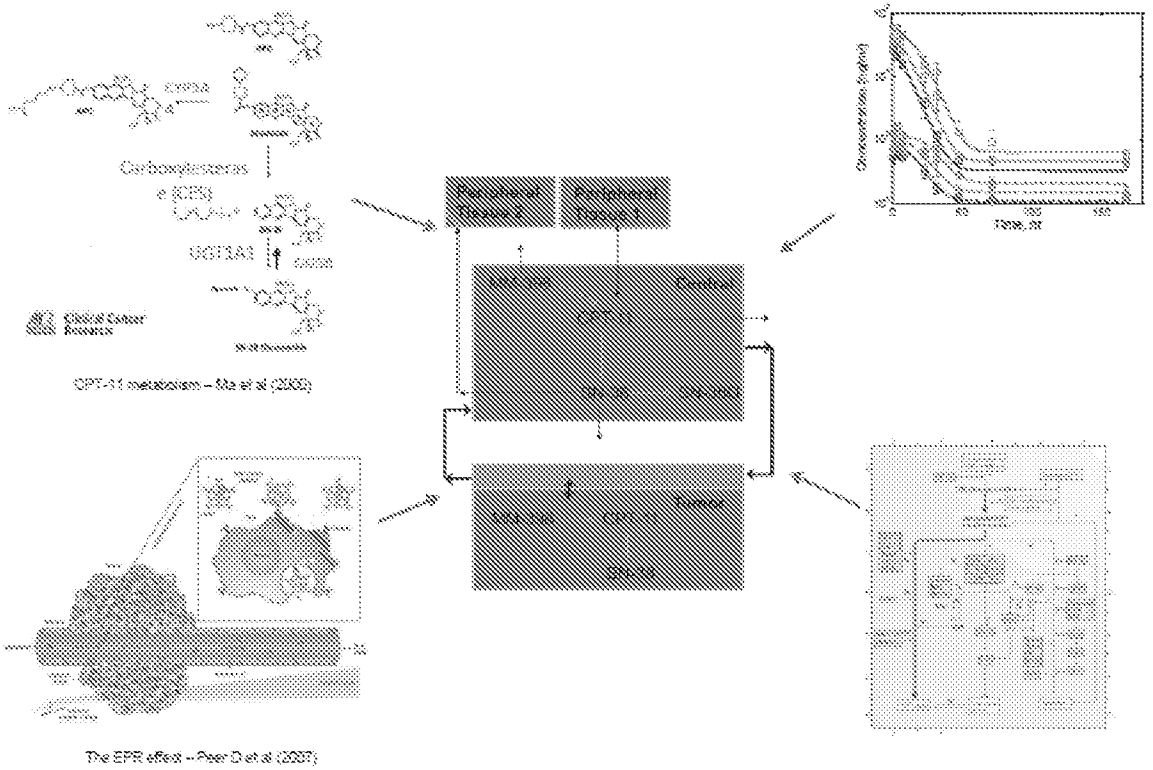


Figure 1. Components to be captured in our mechanism based PK model include the pathway activities for irinotecan metabolism including pharmacogenomic information, deposition parameters for liposomes and free metabolites as part of the Enhanced Permeability and Retention (EPR) effect, measured PK and biodistribution of metabolites in human and mouse as well as considerations of the pathways associated with DNA damage repair and modulation of apoptosis survival as outlined by Rowinsky (2004)

Mechanistic PK model identifies doses of MM-398 and irinotecan (IRI) with equal exposure but not equal activity in mice

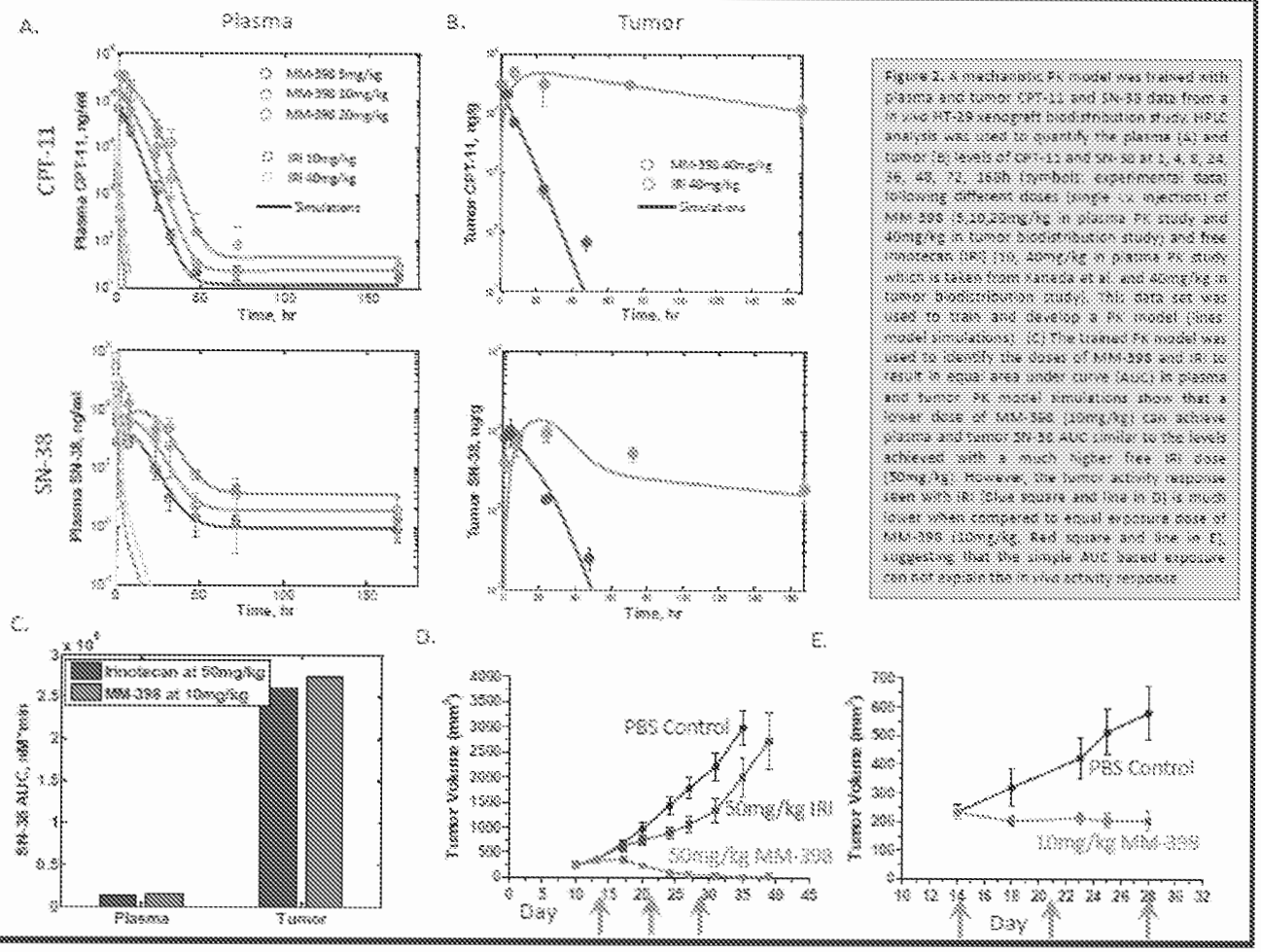


Figure 2. A mechanistic PK model was trained with plasma and tumor CPT-11 and SN-38 data from a in vivo HT-29 xenograft biodistribution study. HPLC analysis was used to quantify the plasma (A) and tumor (B) levels of CPT-11 and SN-38 at 1, 4, 8, 24, 36, 48, 72, 168h (symbol: experimental data) following different doses (single i.v. injection) of MM-398 (5,10,20mg/kg in plasma PK study and 40mg/kg in tumor biodistribution study) and free irinotecan (IRI) (10, 20mg/kg in plasma PK study which is taken from Kameda et al. and 40mg/kg in tumor biodistribution study). This data set was used to train and develop a PK model (lines: model simulations). (C) The trained PK model was used to identify the doses of MM-398 and IRI to result in equal area under curve (AUC) in plasma and tumor. PK model simulations show that a lower dose of MM-398 (10mg/kg) can achieve plasma and tumor SN-38 AUC similar to the levels achieved with a much higher free IRI dose (50mg/kg). However, the tumor activity response seen with IRI (Blue square and line in D) is much lower when compared to equal exposure dose of MM-398 (10mg/kg, Red square and line in E), suggesting that the simple AUC based exposure can not explain the in vivo activity response.

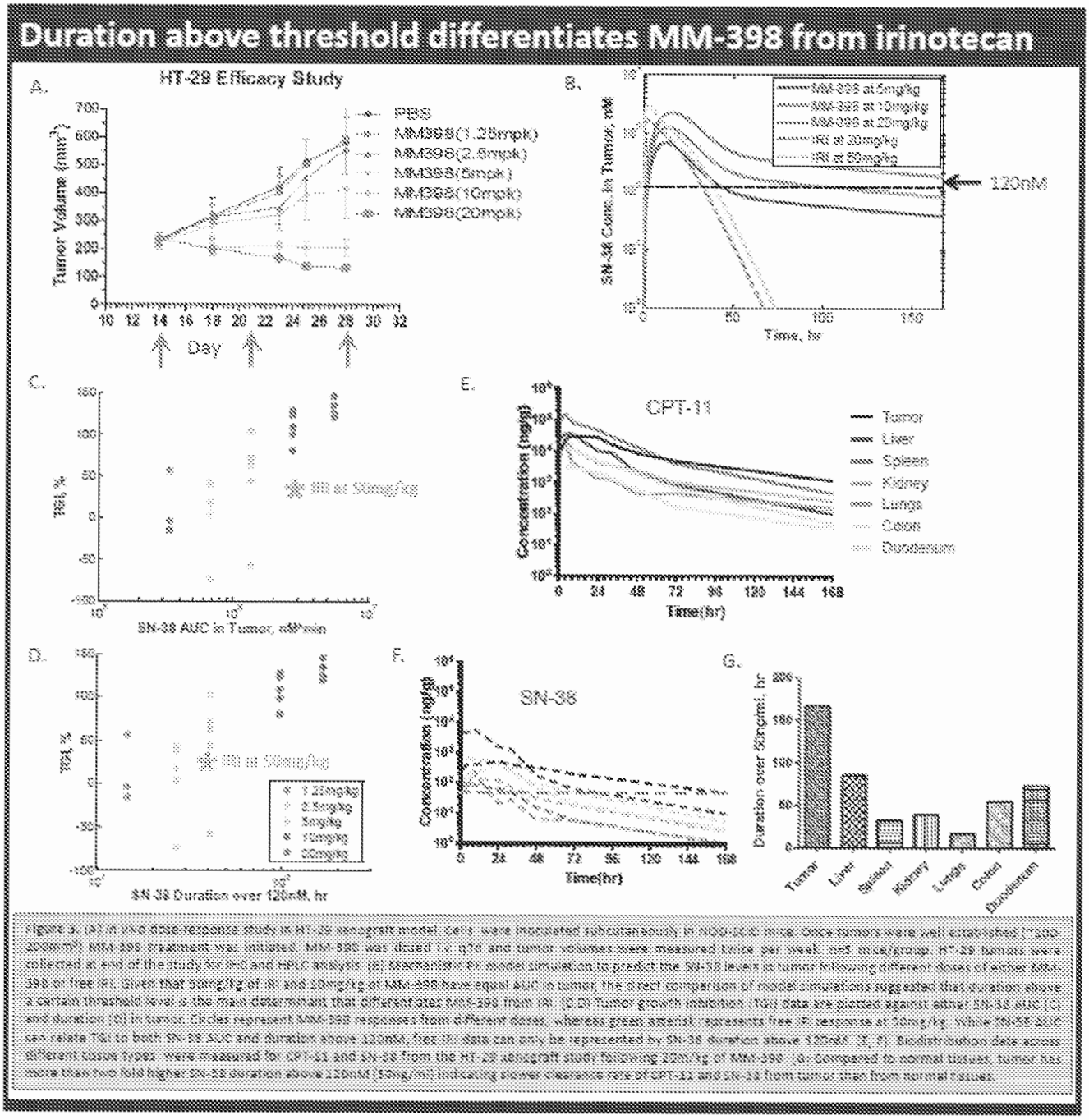


Figure 3. (A) In vivo dose-response study in HT-29 xenograft model. Cells were inoculated subcutaneously in NOD-SCID mice. Once tumors were well established (~100-300mm³) MM-398 treatment was initiated. MM-398 was dosed iv. q7d and tumor volumes were measured twice per week. n=5 mice/group. HT-29 tumors were collected at end of the study for IHC and HPLC analysis. (B) Mechanistic PK model simulation to predict the SN-38 levels in tumor following different doses of either MM-398 or free IRI. Given that 50mg/kg of IRI and 10mg/kg of MM-398 have equal AUC in tumor, the direct comparison of model simulations suggested that duration above a certain threshold level is the main determinant that differentiates MM-398 from IRI. (C,D) Tumor growth inhibition (TGI) data are plotted against either SN-38 AUC (C) and duration (D) in tumor. Circles represent MM-398 responses from different doses, whereas green asterisk represents free IRI response at 50mg/kg. While SN-38 AUC can relate TGI to both SN-38 AUC and duration above 120nM, free IRI data can only be represented by SN-38 duration above 120nM. (E, F) Biodistribution data across different tissue types were measured for CPT-11 and SN-38 from the HT-29 xenograft study following 20mg/kg of MM-398. (G) Compared to normal tissues, tumor has more than two fold higher SN-38 duration above 120nM (50ng/ml) indicating slower clearance rate of CPT-11 and SN-38 from tumor when from normal tissues.

Mechanistic PK model can be used to understand the relative importance of mouse-specific serum CES compared to tumor CES

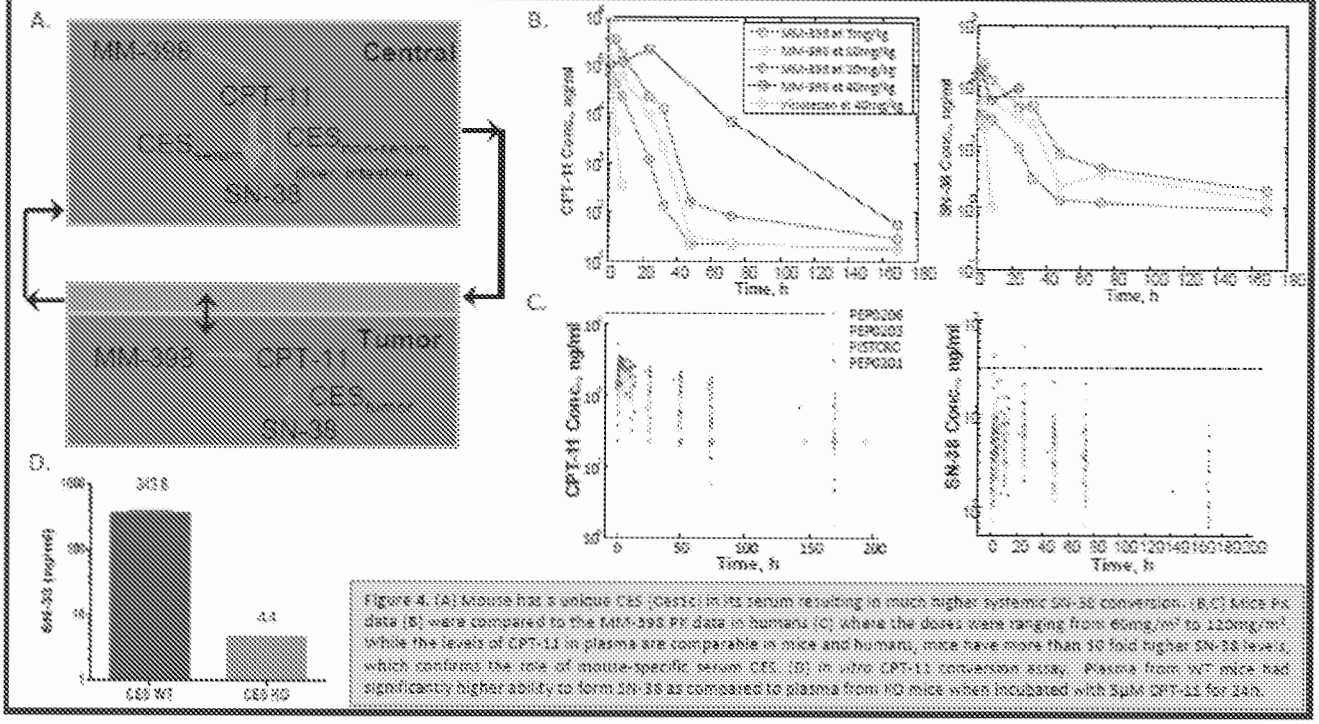


Figure 4. (A) Mouse has a unique CES (Ces1c) in its serum resulting in much higher systemic SN-38 conversion. (B,C) Mice PK data (B) were compared to the MM-398 PK data in humans (C) where the doses were ranging from 60mg/m² to 120mg/m². While the levels of CPT-11 in plasma are comparable in mice and humans, mice have more than 10 fold higher SN-38 levels, which confirms the role of mouse-specific serum CES. (D) In vitro CPT-11 conversion assay. Plasma from WT mice had significantly higher ability to form SN-38 as compared to plasma from KO mice when incubated with 5μM CPT-11 for 1hr.

Tumor CES is responsible for longer duration of SN-38 in tumor even in the mouse system with high serum CES

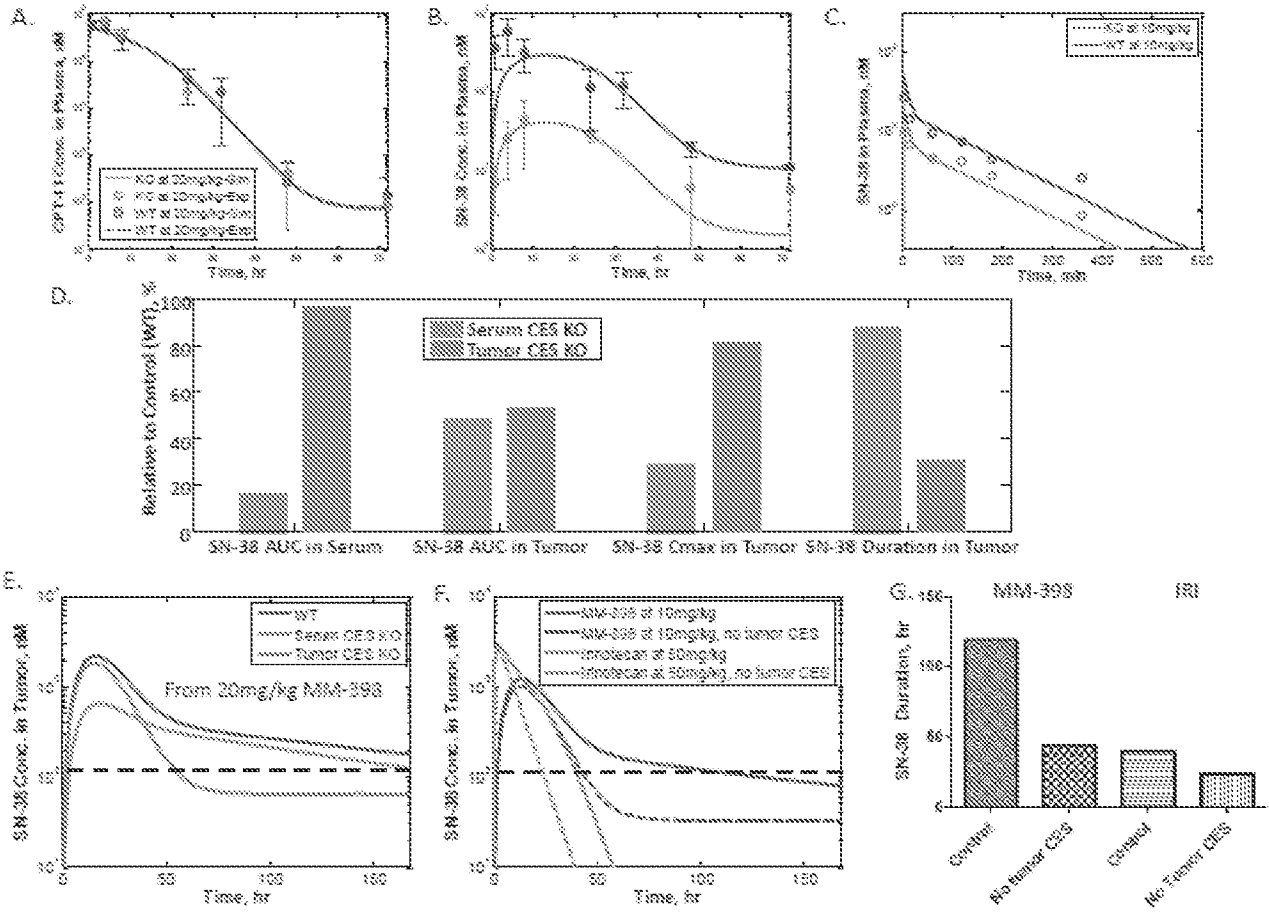


Figure 9. (A,B) in vivo PK data and simulations in WT vs. Ces1c KO mice. Mice bearing LL2 (murine lung carcinoma) were injected i.v. with single dose of MM-398 (20mg/kg) and plasma samples were collected at 1, 4, 8, 24, 48 and 72h. CPT-11 and SN-38 levels in plasma were determined using HPLC (symbols: experimental data; line: model simulations). Plasma profile for CPT-11 was not altered in WT vs. Ces1c KO, whereas the plasma levels of SN-38 lowered ~90 fold in the Ces1c KO mice suggesting significant contribution of plasma CES to CPT-11 systemic conversion in mice. The estimated parameters suggested that ~85% of CPT-11 conversion is due to the serum CES (Ces1c) in mice. (C) By assuming 85% contribution by serum CES, free IRI PK model was used to predict SN-38 levels in both WT and CES knockdown mice (Ces1c) showing a good agreement with the PK data from Morton et al. (D,E) Modeling the effects of CES KOs in serum and tumor on SN-38 exposures (AUC, Cmax and duration). Systemic CPT-11 conversion reduced ~85% with serum CES KO with limited effect by tumor CES KO. Serum and tumor CES KO had similar effects on tumor SN-38 levels (50% less compared to WT). However, the tumor CES KO had a more pronounced effect of reducing the SN-38 duration (~70%) within the tumor compared to WT, whereas SN-38 Cmax in tumor is more affected by serum CES KO. Even though the model simulations are for mouse CESs, serum CES KO simulation in Figure 9.E could be close to human response. (F,G) Comparison of MM-398 and IRI on the effect of tumor CES KO. When the equal exposure doses of MM-398 and IRI were compared, knocking out tumor CES has greater effect in MM-398 (~120hr vs ~40hr) than in IRI (~40hr vs ~30hr). This indicates that the local conversion of CPT-11 is the major driving force for MM-398 in vivo activity.

CES activity and MM-398 permeability modulate SN-38 duration

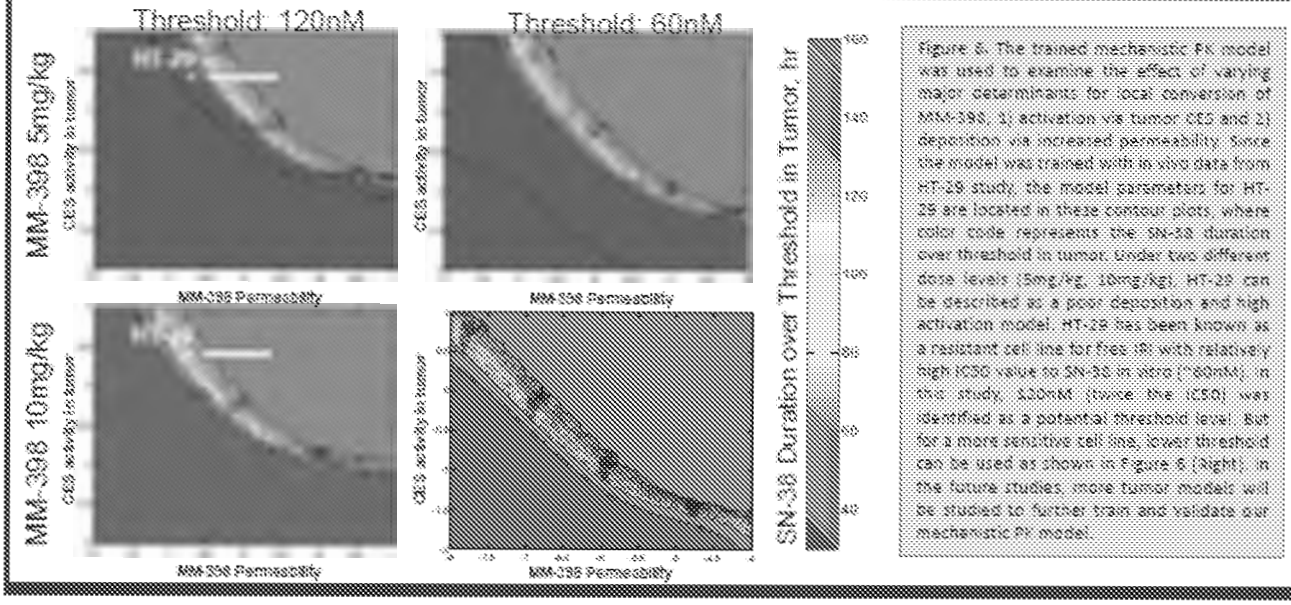


Figure 6. The trained mechanistic PK model was used to examine the effect of varying major determinants for local conversion of MM-398, 1) activation via tumor CES and 2) deposition via increased permeability. Since the model was trained with in vivo data from HT-29 study, the model parameters for HT-29 are located in these contour plots, where color code represents the SN-38 duration over threshold in tumor, under two different dose levels (5mg/kg, 10mg/kg). HT-29 can be described as a poor deposition and high activation model. HT-29 has been known as a resistant cell line for free IRI with relatively high IC50 value to SN-38 in vitro (~60nM). In this study, 120nM (twice the IC50) was identified as a potential threshold level. But for a more sensitive cell line, lower threshold can be used as shown in Figure 6 (right). In the future studies, more tumor models will be studied to further train and validate our mechanistic PK model.

Summary

- Developed mechanistic PK model that correctly simulates MM-398 and free CPT-11 PK in plasma and tumor
- Duration over threshold in tumor may more accurately predict the in vivo response from both MM-398 and IRI
- Model predicted local conversion of CPT-11 is the main driver for extended duration of SN-38 in tumor from MM-398
  - Limited effect of serum CES on the tumor response from MM-398
- More tumor models will be screened for activation and deposition parameters to further train and validate our mechanistic PK model

Sustained intratumoral activation of MM-398 results in superior activity over irinotecan demonstrated by using a systems pharmacology approach
MERRIMACK PHARMACEUTICALS
Jaeyeon Kim, Ashish Kabra, Milind Chakhatan, Stephan Klinz, Nancy Paz, Bart Hendriks, Daryl Drummond, Dmitri Kirpotin, Victor Moyo, Eliel Bayever, Peter Lewins, Chet Mykietyn, Ulrik Nielsen, Jonathan Fitzgerald
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Identifying differential mechanisms of action for MM-398/PEP02, a novel nanotherapeutic encapsulation of irinotecan

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#C207

ABSTRACT

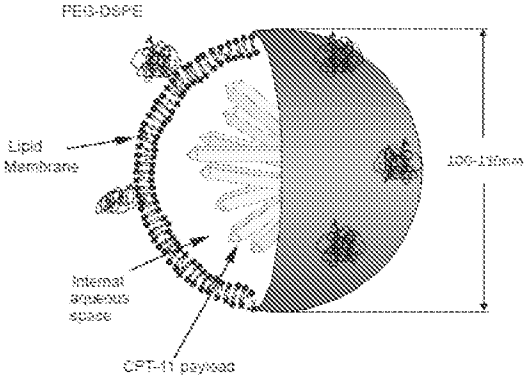
MM-398 (aka PEP02) is a stable, nanotherapeutic encapsulation of the pro-drug CPT-11 (irinotecan) that is currently in clinical development. In preclinical experiments, treatment with MM-398 resulted in significantly higher intratumoral concentrations of both irinotecan (142-fold) and its major metabolite, SN-38 (9-fold) and exhibited superior anti-tumor activity compared to free irinotecan in multiple tumor xenografts. Subsequently, multiple phase 1 and 2 studies have established a pharmacokinetic and safety profile that supports continued clinical development, including in pancreatic, gastric, colorectal and potentially other cancers.

Because current evidence suggests that resistance to pancreatic cancer is driven largely by inadequate drug penetration into these often poorly vascularized, stromally dense and hypoxic tumors, we sought to better understand how this relatively large (100nm) liposomal nanotherapeutic could potentially result in increased efficacy in very advanced gemcitabine-resistant pancreatic cancer and other cancer types.

We developed a mechanism-based PK model of MM-398 and free irinotecan designed to predict intratumor SN-38 levels. Sensitivity analysis revealed that for MM-398 local activation of irinotecan to SN-38 was a far more important parameter than systemic activation. Through cellular uptake studies, we demonstrated *in vitro* that MM-398 was preferentially internalized by phagocytic macrophage/monocyte cell lines and, to a far lesser extent, by tumor cell lines. Furthermore, tumor microdistribution studies by flow cytometry and IHC showed uptake of MM-398 liposomes in both tumor cells and tumor-associated macrophages with more liposomal material being present in the macrophages. This distribution also suggests that macrophages may contribute to the postulated rate limiting process of irinotecan activation.

The sensitivity analysis also suggested that tumor permeability and vascularization are important determinants of tumor-associated SN-38 levels for both free irinotecan and MM-398. To determine the effect of MM-398 on these parameters we treated mice bearing HT29 (colorectal cancer) xenografts with a single dose of MM-398 and measured hypoxic markers (CAIX) and microvessel density (CD31) by IHC. Tumors treated with MM-398 showed a greater degree of CD31 staining and lower CAIX staining, indicating that MM-398 may be able to affect tumor characteristics that traditionally have contributed to therapy resistance and limited the delivery of cancer therapeutics.

In summary, encapsulation of irinotecan alters rate-limiting processes that determine tumoral SN-38 levels. Delivery of MM-398 is believed to alter tumor microvessel density and decrease hypoxia. These intriguing mechanism of action findings support the continued clinical development of MM-398 as a differentiated therapeutic for several cancer types.



Systems Pharmacology of MM-398

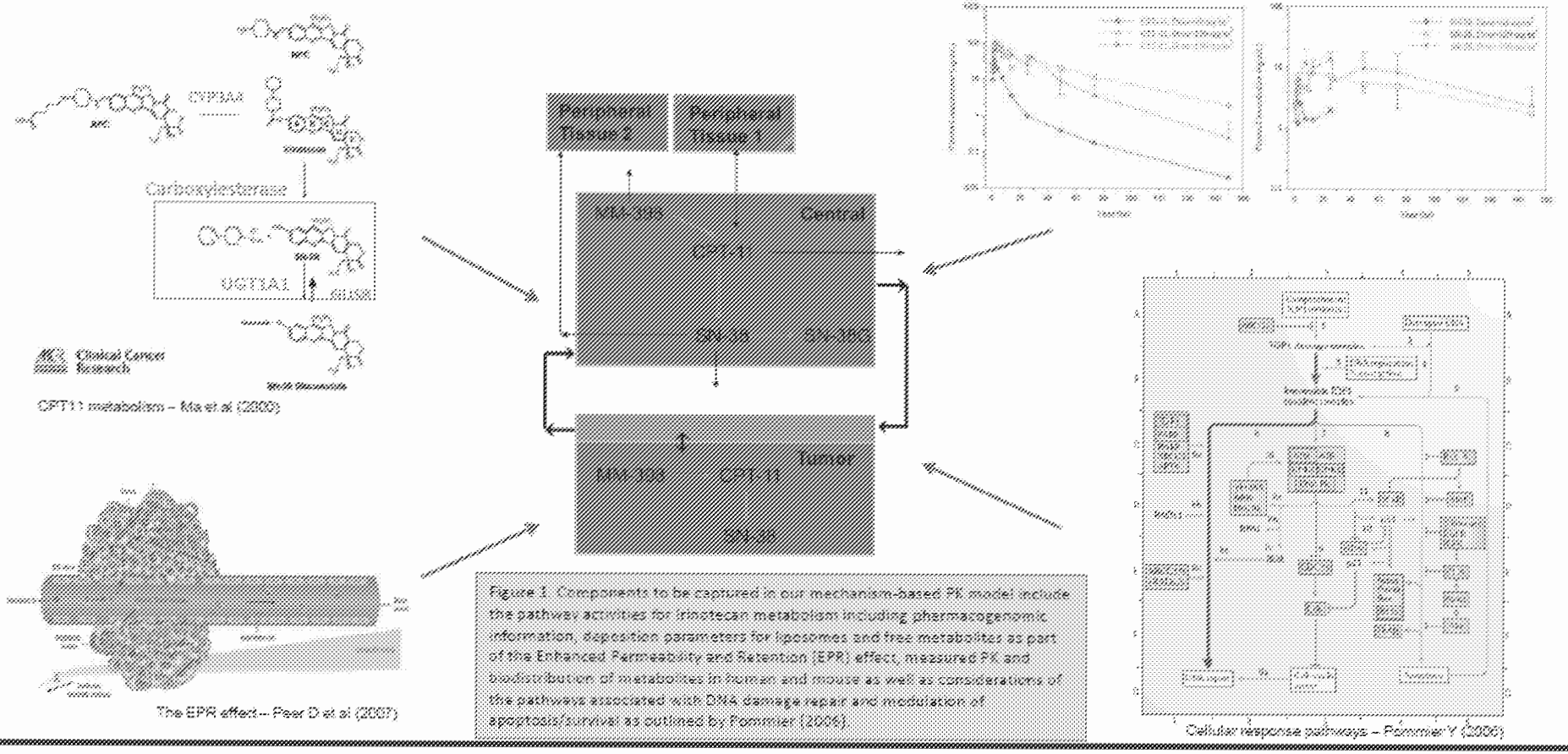
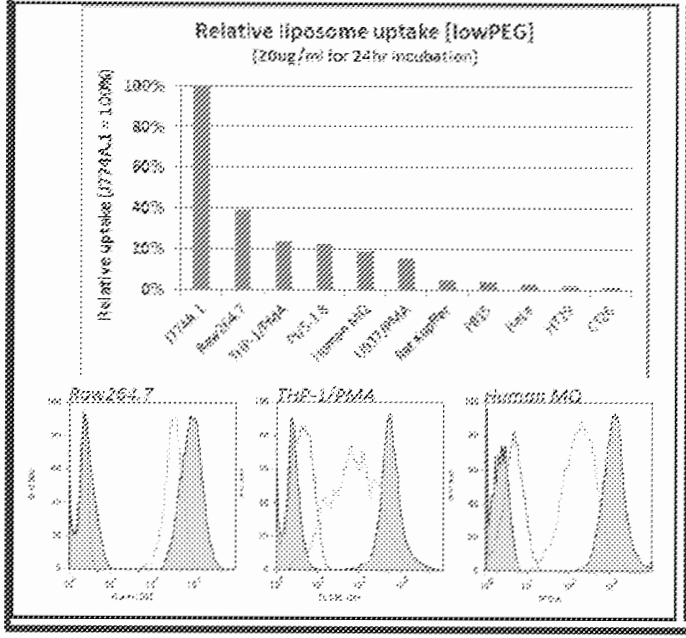


Figure 1. Components to be captured in our mechanism-based PK model include the pathway activities for irinotecan metabolism including pharmacogenomic information, deposition parameters for liposomes and free metabolites as part of the Enhanced Permeability and Retention (EPR) effect, measured PK and biodistribution of metabolites in human and mouse as well as considerations of the pathways associated with DNA damage repair and modulation of apoptosis/survival as outlined by Pommier (2006).

Figure 2. A mechanistic PK model was created with intravenous CPT-11 and SM-38 data from rat in vivo reagent miss study. 20 mg/kg of either MM-398 or free irinotecan was intravenously given to tumor-bearing mice. CPT-11 and SM-38 levels in tumor were assessed at either 1, 2, 24, 72, 168h after MM-398 administration or 1, 4, 12, 24, 48h after irinotecan administration. Sensitivity analysis revealed that permeability of MM-398 (depicted as $PeOUT_{MM398}$) and CES activity in tumor (depicted as $CES3a_{Tumor}$) are most important parameters for intratumoral SM-38 exposure. The effect of systemic and intratumoral CES activities on intratumoral SM-38 were compared for MM-398 and irinotecan. MM-398 exposure is only affected by intratumoral CES activity. The fact that CES activity in tumor is not a rate limiting step in MM-398 exposure supports the significance of local conversion of CPT-11 in response to MM-398.

Figure 3 – In vivo deposition of liposomes. Liposomal DiI-based markers are used in frozen sections of HT-29 tumors 24hr after injection. The liposomes are observed to be associated with cellular and interstitial components, allowing analysis of tumor-derived cells by flow cytometry after enzymatic and mechanical dissociation techniques. Liposome signal in both tumor cell and macrophage cell populations is observed. Frequency of recovered tumor cells, volume mean size of the liposomes, and relative brightness of higher order assemblies in the HT-29 tumor model are nearly identical to previous observations with EPC/Chol-positive tumor cells dosed with liposome-based drug delivery systems.

Phagocytic Cell Lines Preferentially Take Up Untargeted Liposomes



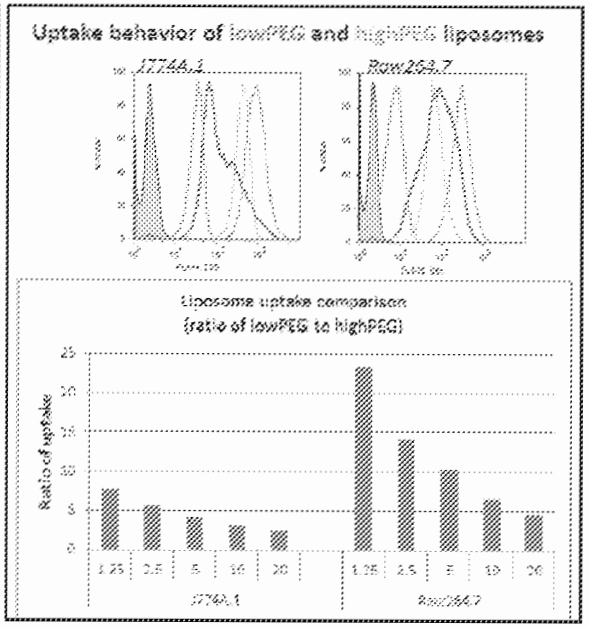


Figure 4 – Cellular uptake behavior of liposomes: (Left) DiI5-labeled liposomes were incubated with various cell lines (ATCC) or primary cells (Rat Kupffer Cells [Invitrogen]; Human Monocyte-derived Macrophages [StemCell Technologies]) for 24hrs at 20μg/ml. Flow cytometry was performed on a BD FACS Calibur instrument. Mean MFI values for the FL4H/APC channel were calculated and expressed relative to J774A.1 cells, which showed the highest uptake rate for untargeted liposomes compared to other cell lines. Histograms for three cell preparations show the DiI5 intensity distribution with J774A.1 (blue-tinted histogram) as comparator. (Middle) DiI5-labeled liposomes with low or high pegylation levels (corresponding to MM-398 and Doxil, respectively) were incubated with cells at 1.25 – 20μg/ml drug concentration for 24hrs. Liposomes show higher uptake particularly at low (bold histogram – 1.25μg/ml) compared to high concentrations (thin histogram – 20μg/ml).

In Vivo Activity of MM-398

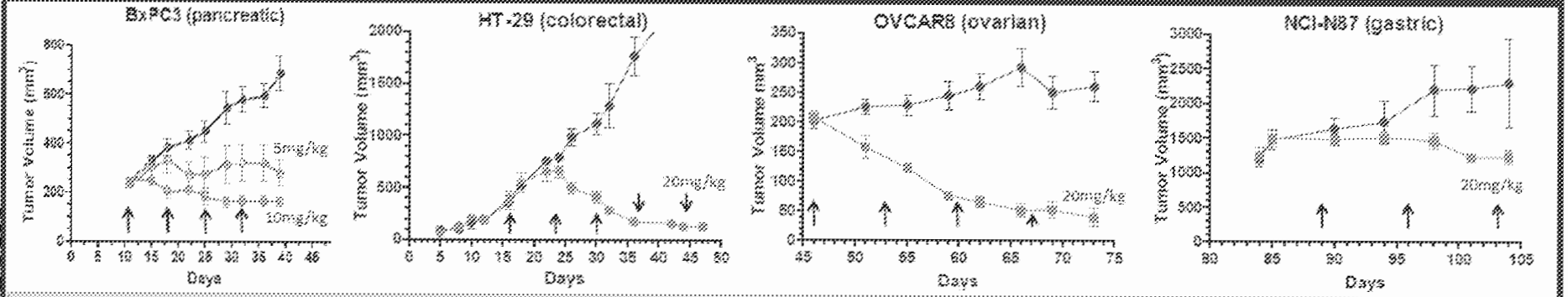


Figure 5 – *In vivo* xenograft activity studies: Different tumor cell lines (BxPC3, HT-29, OVCAR8, NCI-N87) were inoculated subcutaneously in SCID mice. Once tumors were well established (~100-500mm³) MM-398 treatment was initiated. MM-398 (red) was dosed at the indicated concentrations and times (arrows). Control tumors (blue) received saline injections. Tumor volumes were measured twice per week. N=4-6 mice/group.

Figure 6. (A-H) HT-29 tumors were isolated 5 days after a injection of NAB-SN-38 (MM-398) corresponding to the earliest time point when tumor recovery resumed growth trajectory. Tissue sections were stained with (A,B) anti-CAIX, (C,D) anti-CD31, (E,F) anti-Ki67, and (G,H) anti-cleaved caspase-3 antibodies. Representative images are shown. Magnification, ×20. (I-K) Quantification of CAIX staining, CD31 staining, and Ki67 staining, respectively. Data are presented as the mean ± SEM. Statistical significance was determined by Student's t test. *P<0.05, **P<0.01. (L) Quantification of cleaved caspase-3 staining. Data are presented as the mean ± SEM. Statistical significance was determined by Student's t test. *P<0.05. (M-O) Quantification of CAIX staining, CD31 staining, and Ki67 staining, respectively. Data are presented as the mean ± SEM. Statistical significance was determined by Student's t test. *P<0.05, **P<0.01. (P) Quantification of cleaved caspase-3 staining. Data are presented as the mean ± SEM. Statistical significance was determined by Student's t test. *P<0.05. (Q) Quantification of CAIX or CD31 marker area coverage normalized to total tissue nuclear area.

Current Clinical Activities

- Planned Phase 3 monotherapy in 2nd line pancreatic cancer
- Ongoing Phase 2 combination with 5-FU & LV in 2nd line colorectal cancer
- Ongoing Phase 2 monotherapy in 2nd line pancreatic cancer
- Ongoing Phase 1 monotherapy in 2nd and 3rd line colorectal cancer
- Completed Phase 2 monotherapy in 2nd line gastric cancer

SUMMARY

- A mechanism-based PK model for MM-398 has been developed.
- The model suggests that local conversion of irinotecan to SN-38 is an important parameter.
- MM-398 is preferentially taken up by phagocytic monocyte/macrophages in vitro and in vivo.
- MM-398 shows activity in multiple tumor models, including the hypoxic HT-29 model.
- MM-398 induces changes in the vascular microenvironment of tumors as evidenced by changes in CAIX and CD31 levels.

Acknowledgments

We thank foremost the patients in the initial clinical trials that have yielded insight into the pharmacokinetic behavior of MM-398 and allowed continued clinical development of this drug. We thank Gabriela Garcia and Aaron Fulgham for contributing the effect of MM-398 in the BxPc3 tumor activity model. Violette Paragas and Elena Garetti helped with the implementation of the Aperio ScanScope FL and the use of the Definiens analysis software.

#C207

Identifying differential mechanisms of action for MM-398/PEP02, a novel nanotherapeutic encapsulation of irinotecan

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MERRIMACK
PHARMACEUTICALS

Abstract

Purpose: To investigate the mechanisms of action for MM-398/PEP02, a novel nanotherapeutic encapsulation of irinotecan.

Methods: MM-398 was compared to irinotecan in vitro and in vivo. In vitro, cytotoxicity was assessed in a panel of human cancer cell lines. Cell cycle effects were evaluated by flow cytometry. In vivo, efficacy was evaluated in human tumor xenograft models. Pharmacokinetics and tissue distribution were assessed in tumor-bearing mice. Pharmacodynamic effects were evaluated by immunohistochemistry.

Results: In vitro, MM-398 showed cytotoxicity in a panel of human cancer cell lines. MM-398 induced cell cycle arrest and apoptosis. In vivo, MM-398 showed dose-dependent antitumor activity in multiple xenograft models. MM-398 showed prolonged circulation and tumor accumulation of irinotecan and SN-38 compared to irinotecan. MM-398 showed increased DNA damage and apoptosis in tumor tissue.

Conclusions: MM-398 showed differential mechanisms of action compared to irinotecan, including prolonged exposure to irinotecan and SN-38, increased tumor accumulation, and increased DNA damage and apoptosis in tumor tissue.

Introduction

Methods

Results

Conclusions

References

Acknowledgements

FULL PAPER

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Keywords: irinotecan sucrosofate; liposomes; pancreatic cancer; second-line; gemcitabine-refractory

A multinational phase 2 study of nanoliposomal irinotecan sucrosofate (PEP02, MM-398) for patients with gemcitabine-refractory metastatic pancreatic cancer

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Background: PEP02, also known as MM-398, is a novel nanoliposomal irinotecan that has improved pharmacokinetics and tumour bio-distribution of the free drug. This phase 2 study evaluated PEP02 monotherapy as second-line treatment for pancreatic cancer.

Methods: Patients who had metastatic pancreatic adenocarcinoma, Karnofsky performance status $\geqslant 70$, and had progressed following gemcitabine-based therapy were eligible. Intravenous injection of PEP02 120 mg m^{-2} was given every 3 weeks. Simon 2-stage design was used. The primary objective was 3-month survival rate ($OS_{3\text{-month}}$).

Results: A total of 40 patients were enrolled. The most common severe adverse events included neutropenia, abdominal pain, asthenia, and diarrhoea. Three patients (7.5%) achieved an objective response, with an additional 17 (42.5%) demonstrating stable disease for a minimum of two cycles. Ten (31.3%) of 32 patients with an elevated baseline CA19-9 had a $>50\%$ biomarker decline. The study met its primary end point with an $OS_{3\text{-month}}$ of 75%, with median progression-free survival and overall survival of 2.4 and 5.2 months, respectively.

Conclusion: PEP02 demonstrates moderate antitumour activity with a manageable side effect profile for metastatic, gemcitabine-refractory pancreatic cancer patients. Given the limited treatment options available to this patient population, a phase 3 trial of PEP02 (MM-398), referred to as NAPOLI-1, is currently underway.

Therapeutic options for patients with advanced pancreatic cancer (APC) range from gemcitabine monotherapy to multiple-drug regimens, depending on age, performance status, comorbid conditions, and patient and physician preference. Recently, results of a phase 3 clinical trial from France (PRODIGE 4/ACCORD 11) demonstrated the superiority of FOLFIRINOX (biweekly infusional 5-fluorouracil, leucovorin, irinotecan, and oxaliplatin) over gemcitabine in the first-line treatment of metastatic pancreatic

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cancer, with improvements in response rate, progression-free survival, and overall survival, albeit with greater toxicity (Conroy *et al*, 2011).

Beyond first-line therapy, options for metastatic pancreatic cancer become less clear, as patients often demonstrate rapid clinical deterioration and are no longer suitable candidates for additional treatment beyond best supportive care. One co-operative group trial reported that only 45% of patients with APC went on to receive additional therapy following progression on front-line study treatment (Schrag *et al*, 2007). A number of small prospective single-arm studies have evaluated both cytotoxic and/or targeted agents in the setting of gemcitabine-refractory disease, generally demonstrating low response rates and progression-free survival of a few months at best (Burris *et al*, 2005; Boeck *et al*, 2007; Kulke *et al*, 2007; Ko *et al*, 2008, 2010; Oh *et al*, 2010; O'Reilly *et al*, 2010). Results from a randomised German trial for the second-line treatment of APC (CONKO-003) suggested a weekly regimen called OFF (oxaliplatin, 5-FU given as a 24-hour infusion, and folinic acid) may improve patient outcomes in patients refractory to gemcitabine (Pelzer *et al*, 2008, 2011). At present, however, there is no recognised standard of care in this setting.

PEP02 (also known as MM-398) is irinotecan sucrosofate encapsulated in a liposome drug delivery system. This stable nanoliposomal formulation has been shown in preclinical studies to improve pharmacokinetics and tumour bio-distribution of both irinotecan and its active metabolite SN-38 when compared with the free form of the drug, with less accumulation in many of the target organs associated with toxic side effects. PEP02 also demonstrated increased efficacy and tolerable toxicity when compared with free irinotecan in an orthotopic pancreatic cancer mouse model (Hann *et al*, 2007). The favourable pharmacokinetics of irinotecan and SN-38 after PEP02 was confirmed in the first-in-human phase 1 trial for refractory solid tumours, in which the maximum tolerated dose of PEP02 given every 3 weeks was determined as 120 mg m^{-2} (Chen *et al*, 2008). This non-randomised phase 2 trial, conducted in the United States and Taiwan, sought to establish the efficacy and toxicity of single-agent PEP02 in patients with metastatic pancreatic cancer after progression on first-line gemcitabine-based therapy.

PATIENTS AND METHODS

Trial design and patients. This trial was an international, multicenter, open-label, phase 2 study of PEP02 (liposome encapsulated irinotecan, PharmaEngine Inc, Taipei, Taiwan) in patients with gemcitabine-based chemotherapy failure metastatic pancreatic adenocarcinoma.

Patients with histologically confirmed adenocarcinoma of the exocrine pancreas refractory to gemcitabine-based (either alone or in combination) systemic chemotherapy, including those with disease progression within 6 months after post operative adjuvant therapy, were eligible. Prior treatment with irinotecan was not allowed. Further inclusion criteria were age $\geqslant$18 years, Karnofsky performance status of $\geqslant$50 (subsequently amended to $\geqslant$70 to ensure patient safety and to be consistent with the eligibility criteria of other clinical trials for this same patient population), with extrapancreatic metastases diagnosed either radiographically or by biopsy confirmation, and adequate bone marrow and hepatic functions within 1 week before commencing treatment (absolute neutrophil count $\geqslant 1.5 \times 10^3$ ml^{-1}, platelets $\geqslant 100 \times 10^3$ ml^{-1}, serum bilirubin within upper limit of normal (ULN), transaminase $\leqslant 2.5 \times$ ULN ($\leqslant 5 \times$ ULN in patients with liver metastases). All prior major surgery, radiotherapy (except palliative), or investigational drug therapy, had to be ceased at least 4 weeks and all treatment-related toxicities had to be resolved to no greater than grade 1 before enrolment. Patients with central nervous system metastases, pregnancy, uncontrolled active infection, another primary malignancy within the past 5 years except curatively treated non-melanoma skin cancer or cervical carcinoma *in situ*, or other concomitant serious diseases, were excluded.

All patients gave written informed consent. The trial was approved by the independent ethics committee of each participating institute, and performed in accordance with the International Conference on Harmonization Good Clinical Practice guidelines, Good Clinical Laboratory Practice, and the Declaration of Helsinki. The trial was also registered with clinical trials.gov identifier NCT00813163.

Treatment and assessments. PEP02 at a dose of 120 mg m^{-2} was diluted in 500 ml of 5% dextrose and delivered as a 90-min intravenous infusion every 21 days. Infusion time was allowed to be prolonged for acute infusion-associated reactions or any other clinical needs. Premedication included dexamethasone and a serotonin antagonist. Prophylactic anticholinergic agent was not given unless an acute cholinergic reaction was observed during a prior cycle of treatment. Imodium, growth factor support, and anticoagulation (warfarin or low-molecular heparin) were allowable per protocol as clinically indicated, but not for primary prophylaxis. Detailed history evaluation, vital signs recording, physical examination, complete blood count with differential classification, and blood biochemistry tests were performed weekly during the first treatment cycle and before the start of each treatment cycle thereafter. Toxicity was recorded according to the National Cancer Institute-Common Toxicity Criteria (NCI-CTC) version 3.0.

Dose adjustments in PEP02 were made according to toxicities observed with each treatment cycle. The protocol allowed, at the discretion of the treating physician, escalation of PEP02 to 150 mg m^2 beginning with cycle no. 2 in patients who did not experience drug-related toxicities worse than grade 1. The development of grade 3 or 4 diarrhoea, grade 4 or febrile neutropenia, or any other grade 3 or 4 toxicity required a dose reduction of study drug in 20 mg m^{-2} decrements, to a lowest dose level permissible of 80 mg m^{-2}, with no subsequent dose re-escalation allowed. The treatment was continued until evidence of disease progression, unacceptable toxicity, treatment delay for $>$2 weeks, patient withdrawal of consent, or death.

Imaging studies, preferably using computed tomography, were performed at baseline and after every two cycles of chemotherapy to evaluate tumour response, which was determined according to the RECIST version 1.0 guidelines. All complete and partial responses required confirmation by two consecutive observations no less than 4 weeks apart. CA19-9 was measured before each cycle of treatment, and CA19-9 tumour marker response (defined as a decrease of $\geqslant$50% of CA 19-9 in relation to baseline level at least once during the treatment period, in patients with baseline values above the ULN) was determined. Patient diaries were dispensed to collect pain information (including pain intensity and morphine consumption). Patients' survival status was tracked at the 90th day after the start of PEP02 treatment (cycle 1, day 1) and every 2 months after withdrawal. The date of death was recorded.

Statistical analysis. The primary end point of this study was 3-month survival rate ($OS_{3\text{-month}}$). Secondary end points included other clinical efficacy variables (objective tumour response, progression-free and overall survival, clinical benefit response (as defined in Burris *et al*, 1997), CA19-9 tumour marker response), and safety profile. A randomised phase 3 trial by the German CONKO-study group (Pelzer *et al*, 2011) reported a median survival of 2.3 months in patients receiving best supportive care after front-line gemcitabine-based therapy, with a $OS_{3\text{-month}}$ of $\sim$35%. Thus, for the current study, we used as the null hypothesis (H_0) and alternative hypothesis (H_a) a $OS_{3\text{-month}}$ of 40% and 65%, respectively. The study used an optimal Simon 2-stage design.

With a significance level of $\alpha = 0.05$ and a type 2 error $\beta = 0.10$, if 8 of the first 16 patients enrolled in the first stage reached the 3-month survival time-point, an additional 23 patients would be enrolled in the second stage. At least 21 of the 39 patients were required to survive 3 months or longer to allow rejection of the null hypothesis. A safety stopping rule would be invoked if six or more patients in the first stage experienced grade 3 or 4 diarrhoea.

Descriptive statistics were used for all efficacy variables, with the primary analysis population being the per protocol population (defined as study participants who met all inclusion/exclusion criteria and did not significantly deviate from the study protocol). The frequencies of patients with adverse events were summarised by body system and by major adverse event codes (system/organ/class).

RESULTS

Patient characteristics. Baseline patient characteristics are shown in Table 1. A total of 40 patients were enrolled for the study between March 2009 and September 2010, with an approximately even distribution between US and Taiwanese sites. The majority of patients (60%) had a Karnofsky Performance Score of 90–100 and 77.5% had received a prior gemcitabine-based combination, as opposed to monotherapy, as their first-line regimen. The duration of front-line therapy ranged from 1 to 24 months.

Drug delivery and adverse events. Patients received a mean of 5.875 treatment cycles (range, 1–28 cycles; median 2.5 cycles). Owing to concerns of excess toxicity, primarily asthenia, observed in US patients at the starting dose of 120 mg m^{-2}, the protocol was subsequently amended during the second stage of the study to permit a lower starting dose at 100 mg m^{-2}. In total, 27 of 40 patients (67.5%) on the study were able to be maintained at a dose of 120 mg m^{-2} throughout their entire treatment course, whereas 11 (27.5%) required or initiated therapy at reduced doses. Eleven patients (27.5%) received at least eight treatment cycles. The majority of patients (75%) discontinued study treatment due to disease progression.

The most common toxicities observed during study treatment are shown in Table 2. As expected, gastrointestinal and haematologic toxicities were the most common types seen, as well as fatigue and abdominal pain; these latter symptoms may have been related either to study treatment or to the underlying cancer. In total, 26 patients (65%) experienced at least one treatment-emergent adverse event categorised as grade 3 or higher. Of note, six patients died within 30 days of the last dose of study treatment. Of these, three were attributed to disease progression; the other three were due to respiratory failure, aspiration pneumonia, and sepsis, all in the setting of neutropenia.

Efficacy. Efficacy results are shown in Table 3. Half of the patients (50%) had evidence of disease control (objective response plus stable disease for more than two cycles), including three patients (7.5%) who achieved a confirmed objective response. Fourteen of the 17 patients with stable disease as their best response demonstrated disease stability for at least four cycles (35% of the entire cohort). A waterfall plot (Figure 1) demonstrates best

Table 1. Patient demographics and baseline characteristics

Characteristic	$n = 40$
Sex, *n* (%)	
Male/female	19 (47.5)/21 (52.5)
Age, mean (range) years	58.8 (39–82)
Study site, *n* (%)	
Taiwan/USA	22 (55)/18 (45)
Ethnicity, *n* (%)	
Asian/Caucasian	25 (62.5)/15 (37.5)
Karnofsky performance status, *n* (%)	
100 90 80 70	7 (17.5) 17 (42.5) 6 (15.0) 10 (25.0)
Prior treatment, *n* (%)	
Chemotherapy Radiotherapy Surgery	40 (100) 10 (25.0) 17 (42.5)
First-line chemotherapy and duration in months	
Gemcitabine monotherapy, *n* (%)/median (range) Gemcitabine-based combination, *n* (%)/median (range)	9 (22.5)/2 (1.5–24) 31 (77.5)/6 (1–16)
With elevated CA19-9, *n*	32
Baseline clinical benefit parameters, *n* (%)	
Pain intensity ⩾20 (out of 100) Morphine consumption ⩾10 mg per day	17 (42.5) 14 (35.0)

Table 2. (A) Treatment-emergent adverse events (all grades) occurring in 10% or greater of study patients; (B) Treatment-emergent grades 3–4 adverse events occurring in 10% or greater of study patients

A

Adverse event, all grades	*N* (%)
Diarrhoea	30 (75%)
Fatigue	25 (62.5%)
Nausea	24 (60%)
Anorexia	23 (57.5%)
Vomiting	23 (57.5%)
Alopecia	17 (42.5%)
Neutropenia	16 (40%)
Leucopenia	15 (37.5%)
Abdominal pain	15 (37.5%)
Weight decreased	15 (37.5%)
Anaemia	13 (32.5%)

B

Adverse event, grades 3–4	*N* (%)
Neutropenia	12 (30%)
Leucopenia	10 (25%)
Abdominal pain	6 (15%)
Fatigue/asthenia	8 (20%)
Anaemia	6 (15%)
Hyponatremia	6 (15%)
Diarrhoea	6 (15%)
GGT elevated	5 (12.5%)
Anorexia	4 (10%)
Nausea	4 (10%)

Table 2. Efficacy data

Best tumour response ($n = 40$)	***N* (%)**
Partial response	3 (7.5%)
Stable disease	17 (42.5%)[a]
Disease Progression	10 (25.0%)
Non-evaluable[b]	10 (25.0%)
Disease control (PR + SD) rate	20 (50.0%)
Survival	**Months**
Progression-free survival (median)	2.4
Overall survival (median)	5.2
Proportion of patients alive at:	***N* (%)**
Three months	30 (75%)
Six months	17 (42.5%)
Twelve months	10 (25%)
Clinical benefit response ($n = 25$ evaluable)	5 (20%)
CA19-9 decline >50% ($n = 32$ with elevated level at baseline)	10 (31.3%)

Abbreviations: PR = partial response; SD = Stable disease.
[a]Including eight patients with minor response.
[b]Non-evaluable patients for tumor response included those patients with non-measurable disease at baseline or in whom at least one post treatment radiographic evaluation was not performed.

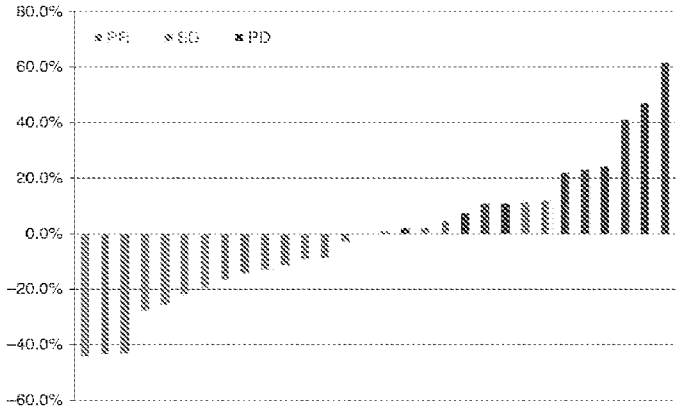

Figure 1. Maximum % change from baseline in sum of target lesion diameters (evaluable patients only, $n = 30$). Abbreviations: PD = disease progression; PR = partial response; SD = stable disease.

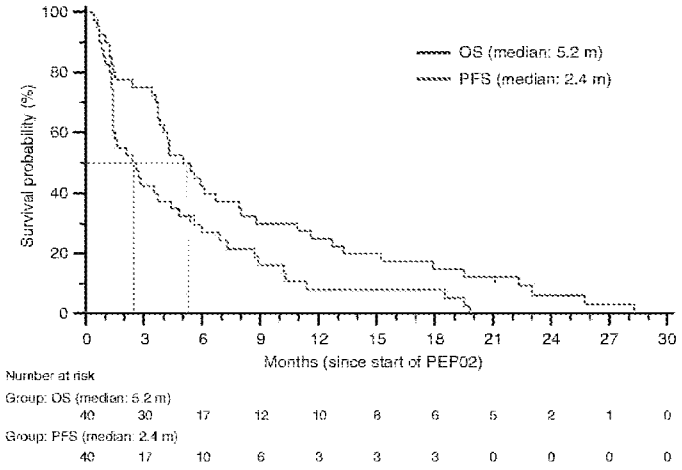

Figure 2. Kaplan–Meier curves of overall and progression-free survival. Abbreviations: m = months; OS = overall survival; PFS = progression-free survival.

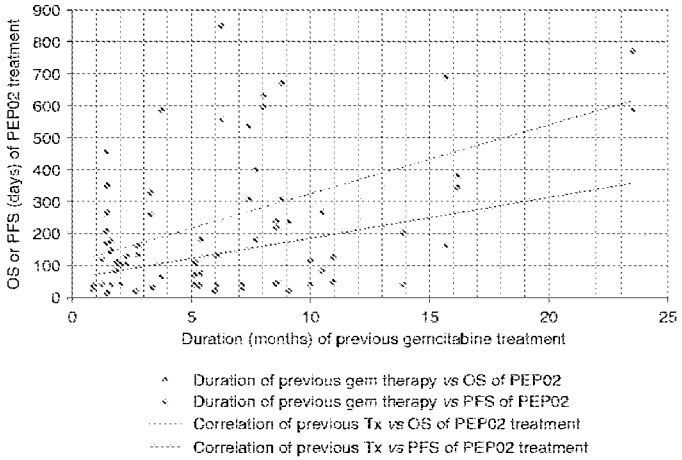

Figure 3. Overall survival (OS) and progression-free survival (PFS) relative to the duration of prior gemcitabine-based therapy. Abbreviations: Gem = gemcitabine; Tx = treatment.

tumour response observed in evaluable study patients. Ten (31.3%) of 32 patients with elevated baseline CA19-9 had >50% biomarker decline, and 5 (20%) of 25 CBR-evaluable patients achieved significant clinical benefit. Median progression-free and overall survival was 2.4 and 5.2 months, respectively (Figure 2). These indicators of antitumour activity are also listed in Table 3. Notably, the study met its primary end point with 75% of patients surviving at least 3 months, including 25% reaching the 1-year mark. Two patients were still alive as of July 2012. Survival outcomes for patients receiving PEP02 showed a modest positive correlation with the duration of prior gemcitabine-based therapy (Figure 3).

DISCUSSION

There is a relative paucity of published studies evaluating the safety and efficacy of chemotherapy regimens in patients with APC who have progressed following first-line therapy. An inherent selection bias is at work in non-randomised trials, as those patients who are well enough to consider salvage treatment may have more favourable tumour biology and a longer survival independent of choice of therapy. Conversely, design of a randomised study in this setting is challenging due to lack of agreement regarding the appropriate selection of control arm; a comparator arm of best supportive care alone, although perhaps appropriate in many cases, is not an appealing option to patients. Results from one of the largest studies conducted to date for the second-line treatment of APC (CONKO-003) randomised 165 patients to receive a weekly regimen called OFF or 5-FU/folinic acid alone (Pelzer *et al*, 2008). Patients receiving the oxaliplatin-containing combination demonstrated significantly improved outcomes in terms of both progression-free survival (13 *vs* 9 weeks, $P = 0.012$) and overall survival (26 *vs* 13 weeks, $P = 0.014$), leading to the adoption of this regimen (or slight variations thereof) as a *de facto* standard of care in the salvage setting.

Irinotecan is a topoisomerase 1 inhibitor that is currently used to treat the colorectal, gastric, lung, uterine, cervical, and ovarian cancers. At higher doses, the drug causes severe diarrhoea and myelosuppression, which is recognised as its dose-limiting toxicity. Specific to pancreatic cancer, irinotecan represents a component of the FOLFIRINOX regimen that has recently demonstrated superior activity to gemcitabine in the front-line setting (Conroy *et al*,

2011), and has also been evaluated as part of combination regimens for refractory disease in several studies (Ko *et al*, 2008; Yoo *et al*, 2009; Gebbia *et al*, 2010; Oh *et al*, 2010; Assaf *et al*, 2011; Zaniboni *et al*, 2012). A recently reported phase 2 trial performed by the Italian Group for the Study of Gastrointestinal Tract Cancer (GISCAD) showed that FOLFIRI produced median progression-free and overall survival rates of 3.2 and 5 months, respectively, in the second-line treatment of APC (Zaniboni *et al*, 2012).

PEP02 is irinotecan encapsulated in a liposome drug delivery system. Liposome drug formulations may reduce the toxicity of an encapsulated agent to healthy tissue while maintaining, or increasing, its antitumour potency. The therapeutic benefits of liposome encapsulated anticancer drugs such as daunorubicin, doxorubicin, and cytarabine are well-established. Preclinical *in vivo* efficacy data have shown improved antitumour activity of PEP02 over the equivalent dose of free irinotecan in multiple established human tumour xenograft mouse models, including brain, colon, and pancreatic cancers (Hann *et al*, 2007). In previous phase 1 studies, PEP02 either alone or in combination with 5-FU/leucovorin demonstrated prolonged disease control in five of seven (71%) patients with gemcitabine-refractory APC (Chen *et al*, 2008, 2010).

On these bases, the current non-randomised phase 2 trial was conducted to establish the preliminary efficacy and safety of PEP02 in the second-line setting for patients with metastatic pancreatic cancer. Recognising the aforementioned limitations that accompany a single-arm study design, PEP02 did show clear evidence of antitumour activity in a subset of patients in whom no standard of care therapy otherwise exists. In addition, although its efficacy profile appears similar to that seen with FOLFIRI in the GISCAD trial for the same patient population, PEP02 may offer advantages in its relative ease of administration as monotherapy without the requirement of an infusion pump. However, it should also be acknowledged that although PEP02 was generally well-tolerated in most patients, with manageable and predictable toxicities, the majority of subjects did experience at least one grade 3 or higher adverse event. In addition, there were three patient deaths that occurred within 30 days of the last dose of study treatment relating to complications of neutropenia. These findings highlight the need to be particularly vigilant with PEP02 (or any cytotoxic therapy, for that matter) in such a fragile patient population, and may support the use of preemptive growth factor support in select patients. Pharmacogenetic testing for polymorphisms in genes relating to the metabolism of PEP02, including UGT1A1 and UGT1A9, was performed on 28 patients; no correlation with either haematologic or non-haematologic toxicity was observed (data not shown).

Although analysis of germline polymorphisms from peripheral blood samples was possible on all study patients, there were not adequate tumour tissue samples available to look for intratumoural molecular biomarkers of potential predictive significance. Such correlative studies represent one of the 'holy grails' that are often attempted to be embedded within pancreatic cancer clinical trials; however, due to scant archived samples and the difficulties in subjecting this patient population to prospective tissue biopsies for research purposes, they continue to present a tremendous challenge in this disease. This obstacle is magnified all the more so in the salvage treatment setting.

The results of this clinical trial are encouraging enough to warrant moving ahead with a larger study in a similar patient population, currently ongoing as an international randomised phase 3 trial called NAPOLI-1 (clinicaltrial.gov. ID: NCT01494506, EudraCT Number: 2011-004687-30). Additional studies may explore this drug's potential role in the first-line setting and as part of combination regimens for APC. Moreover, given the emergence of FOLFIRINOX as a front-line standard in patients with good performance status, the utility of PEP02 in irinotecan-pretreated patients, alone or in combination with gemcitabine, also merits further investigation.

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CONFLICT OF INTEREST

LTC is a consultant and has received honorarium from PharmaEngine. CGY and YWW are employees and hold stock of PharmaEngine, the makers of PEP02. AHK, MAT, YSS, WCS, YLL, ED and AO declares no conflict of interest.

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Nanoliposomal irinotecan (nal-IRI, nal-IRI) population pharmacokinetics (PK) and its association with efficacy and safety in patients with solid tumors

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Introduction

Nanoliposomal irinotecan (nal-IRI, MM-398, PEP02) is irinotecan encapsulated in liposome nanoparticles designed to prolong circulation, enhance delivery, and increase conversion of irinotecan to SN-38 in tumors. In a study evaluating plasma pharmacokinetics (PK) of nal-IRI 120 mg/m^2 and irinotecan HCl 300 mg/m^2 (**Figure 1**), nal-IRI resulted in longer half-lives and higher total irinotecan (tIRI), average (Cavg), and maximum (Cmax) concentrations, while maintaining a lower SN-38 Cmax. In a Phase 3 study in patients with metastatic pancreatic cancer previously treated with gemcitabine (NAPOLI-1), nal-IRI 80 mg/m^2 every 2 weeks in combination with 5-fluorouracil and leucovorin (5-FU/LV) was shown to extend overall survival (OS) compared with 5-FU/LV alone.[2]

The objectives of this study were to quantify plasma population PK of nal-IRI in patients with, to understand the association between baseline covariates and plasma PK, and to evaluate the association between plasma PK with safety (diarrhea and neutropenia) and with the efficacy endpoints in patients with metastatic pancreatic cancer previously treated with gemcitabine (NAPOLI-1 population).

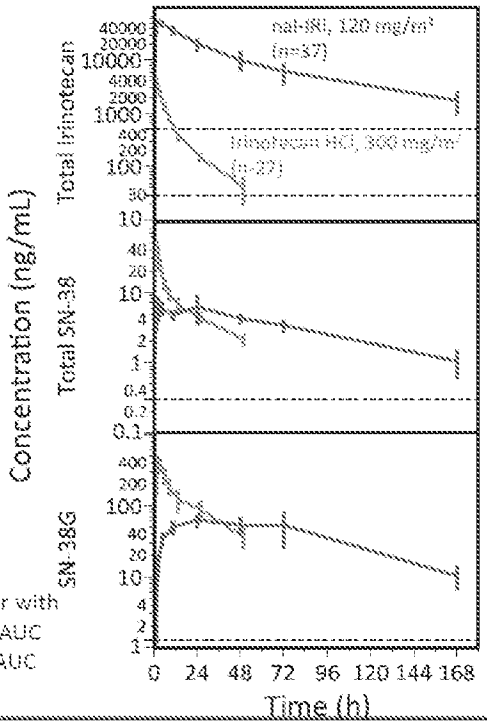

Figure 1. Comparison of plasma PK in patients treated with nal-IRI (n=37) or with irinotecan HCl (n=27). Comparing nal-IRI to irinotecan HCl, total irinotecan AUC was 46 times greater, total irinotecan Cmax was 13.4 times greater, SN-38 AUC was 1.4 times greater, and SN-38 Cmax was 0.19 times greater.[3]

Methods

Population pharmacokinetic analysis of nal-IRI (**Figure 2**) was performed for plasma concentrations of tIRI and its metabolite SN-38 (tSN38) in 353 patients across 6 studies (**Table 1**). The un-encapsulated SN-38 (uSN38) concentration was predicted from the model and appears to be the active metabolite (a fraction of SN-38 was encapsulated inside the liposome and is not bioavailable).[1] SN-38G is the glucuronidated metabolite of SN-38 and is inactive.

PK-safety association was evaluated in a pooled dataset of 353 patients for the most significant adverse-events: neutropenia and diarrhea. PK-efficacy association was evaluated for OS, progression-free survival (PFS) and objective response rate (ORR) in patients from NAPOLI-1.

Figure 2. Population PK model of nal-IRI. Un-encapsulated SN-38 is the active metabolite.

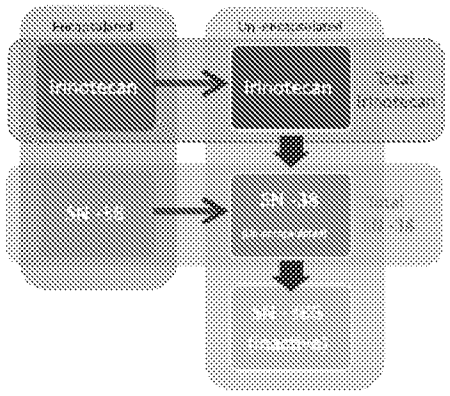

Table 1. Clinical Pharmacology Studies Used in the Population Pharmacokinetic Analysis

Study number	Indication	NAL-IRI dose regimen (mg/m²)	PK sample collections (hours post infusion)	Analytes
PEP0201	Solid tumors (N=11)	60, 120 or 180 every 3 weeks	Cycle 1: predose, 0.5, 1.0, 1.5, 2.5, 3.5, 4.5, 7.5, 10.5, 13.5, 25.5, 49.5, 73.5 and 169.5 Cycle 2: predose	tIRI, encapsulated irinotecan, SN-38
PEP0203*	Solid tumors (N=16)	60, 80, 100 or 120 every 3 weeks	Cycle 1: predose, 0.5, 1.0, 1.5, 2.5, 4.5, 10.5, 25.5, 49.5, 73.5 and 169.5 Cycle 2: predose	tIRI, SN-38
PEP0206†	Gastric and GEJ (N=37)	120 every 3 weeks	Cycle 1: predose, 0.5, 1.0, 1.5, 2.5, 4.5, 10.5, 25.5, 49.5, 73.5 and 169.5 Cycle 2: predose	tIRI, SN-38, SN-38G
PIST-CRC	Colorectal (N=18)	80, 90, 100 every 2 weeks	Cycle 1: predose, 0.5, 1.0, 1.5, 2.5, 4.5, 10.5, 25.5, 49.5, 73.5 and 169.5	tIRI, SN-38
NAPOLI-1*†	Pancreatic cancer (N=260)	Arm 2: 120 every 3 weeks Arm 3: 80 every 2 weeks	Cycle 1: predose, 1.5, 2.5, 48 (Arm 3 only) and 168	tIRI, SN-38, SN-38G, 5-FU
CITS	Solid tumors (N=13)	80 every 2 weeks	Cycle 1: predose, 1.5, 3, 72 and 168 Cycle 2: predose	tIRI, SN-38, SN-38G

*5-FU/LV was used in combination with nal-IRI (Arm 3 in NAPOLI-1). GEJ=gastroesophageal junction. tIRI=total irinotecan. †Only patients who received nal-IRI was included in the analysis.

Patient Characteristics (N=353)

Table 2. Patient Characteristics at Baseline

Characteristic	Subgroup	N (%)
Sex	Male	186 (56)
	Female	167 (44)
Race	Caucasian	182 (52)
	Others	21 (6)
	Asian	150 (42)
Liver Metastasis (NAPOLI-1 only)	No	87 (44)
	Yes	171 (66)
Study	NAPOLI-1	258 (73)
	Others	95 (27)
UGT1A1*28 (NAPOLI-1 only)	Non-7/7	244 (95)
	7/7	14 (5)
Treatment (NAPOLI-1 only)	Nal-IRI+5FU/LV	116 (45)
	Nal-IRI	142 (55)
Tumor Type at Diagnosis	Colorectal	18 (5)
	Gastric and GEJ	37 (10)
	Pancreatic	258 (73)
	Solid tumors	40 (11)
Manufacturing Site	Merrimack	258 (73)
	Other	95 (27)
Initial nal-IRI Dose (mg/m²)	60	4 (1)
	80	141 (40)
	100	11 (3)
	120	187 (53)
	180	4 (1)

Patient characteristics at baseline are listed in **Table 2**. The median age was 63; 56% male; 52% Caucasian and 42% Asian. Patients with hepatic or renal impairment were excluded from the enrollment; 20 patients had bilirubin ≥1 mg/dL (19/20 had bilirubin between 1-2 mg/dL; 1 patient had bilirubin >2 mg/dL). The majority (73%) of the data was obtained from patients with metastatic pancreatic cancer. The majority had an initial dose of 120 mg/m² (53%) or 80 mg/m² (40%).

Table 2 (continued). Patient Characteristics at Baseline

Characteristic	N	Median	5th%	95th %
Age (years)	353	63	35.8	78.2
Albumin (g/dL)	349	4	2.9	4.7
ALT (IU/L)	352	25	8.9	96.3
AST (IU/L)	352	29	14.7	81.9
Bilirubin (umol/L)	352	7	3	19
BSA (m²)	353	1.7	1.3	2.2
CrCl (mL/min)	352	81.3	39.6	151.9

Population Pharmacokinetics Results

A total of 1,800 tIRI samples (355 subjects) and 1,773 tSN38 samples (353 subjects) were analyzed. Typical observed and predicted PK profiles with 80 mg/m^2 and 120 mg/m^2 are shown in **Figure 3**. The time-course of tIRI concentrations were modeled as a two-compartmental model. The time-course of tSN38 were modeled as a one-compartmental model with two input fluxes: from the initial amount of encapsulated SN-38, and from the in vivo conversion of un-encapsulated IRI released from nal-IRI. Compared to 120mg/m^2 every 3 weeks, 80 mg/m^2 every 2 weeks resulted in similar average concentration and 1/3 lower maximum concentration.

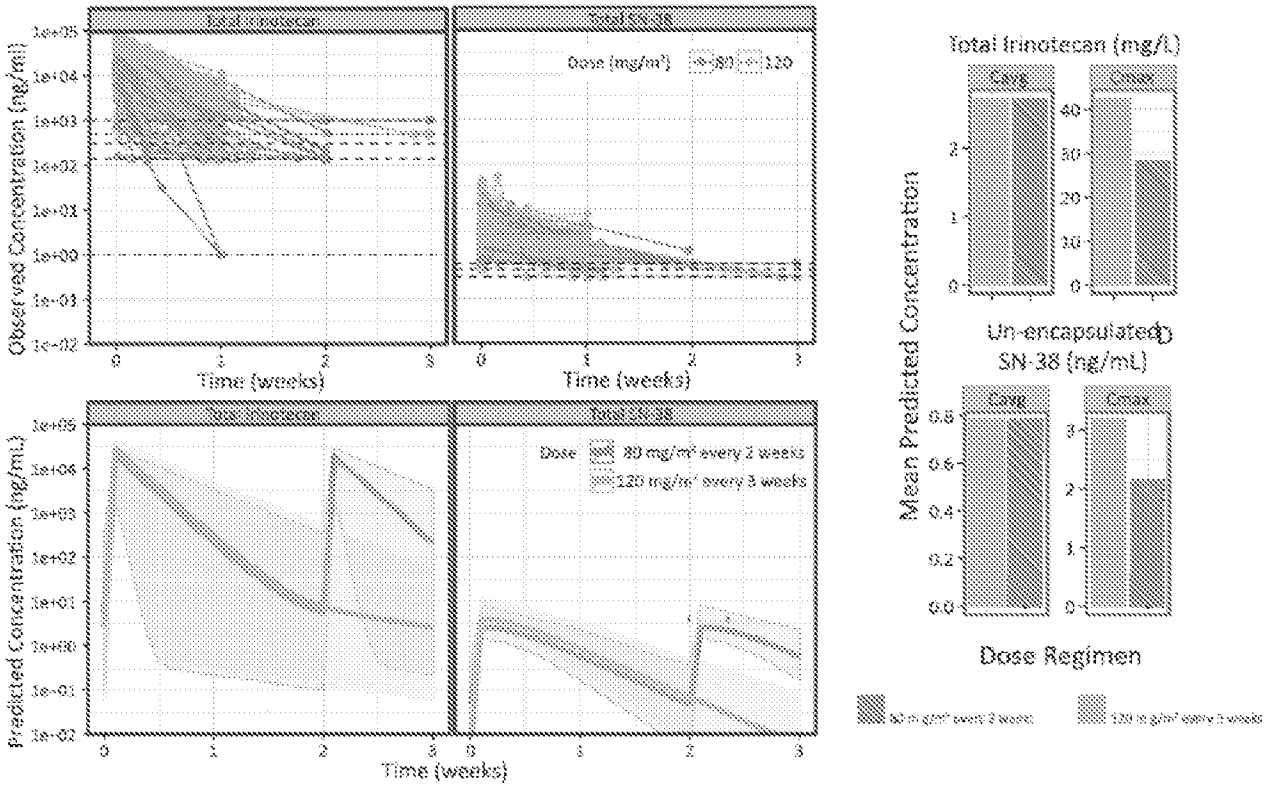

Figure 3. Observed and predicted typical plasma concentration profile of tIRI and SN-38 in patients administered with 80 mg/m^2 every 2 weeks and 120 mg/m^2 every 3 weeks of nal-IRI. A total of 353 patients were used to develop the population PK model. Compared to 120mg/m^2 every 3 weeks, 80 mg/m^2 every 2 weeks resulted in similar average concentration and a 1/3-lower Cmax.

Association between plasma PK and baseline covariates were evaluated for liver metastasis status, total bilirubin, AST, ALT, albumin, creatinine clearance (CrCl), pharmacogenetics (UGT1A1*28), age, sex, race, body surface area (BSA), coadministration with 5-FU/LV, and manufacturing site. Significant or relevant associations are summarized in **Figure 4**. No other significant associations were observed.

Race: Compared to Caucasians, Asians were observed to have lower tIRI and higher SN-38 **(Fig. 4A)**

UGT1A1*28: No significant association was observed. The prevalence of 7/7 homozygosity in Asians were low (1/85[1%]). Compared to non-7/7 Caucasians, 7/7 Caucasians had numerically higher (13%) uSN38 Cmax, but not statistically significant (these numbers were for a simulated dose of 80 mg/m^2 for both homozygous and non-homozygous patients; in NAPOLI-1, the dose in homozygous patients was lower [50 mg/m^2 in nal-IRI+5-FU/LV; 80 mg/m^2 in nal-IRI]). Separate analyses of patients with UGT1A1*28 6/6, 6/7, and 7/7 did not show a significant difference in the clearance of SN-38 (data not shown) for each of the UGT1A1*28 subgroups **(Fig. 4B)**

Bilirubin: Higher baseline bilirubin was associated with higher SN-38 concentration **(Fig. 4B)**

BSA: For tIRI, no association was observed with BSA; for SN-38, increased BSA was associated with lower Cmax. Simulation predicted that, compared to flat-dosing of 136 mg (the nominal dose for a subject with median BSA), a BSA-based dosing strategy would result in lower SN-38 PK variability (interquartile-range of 59% vs 74%) **(Fig. 4C)**

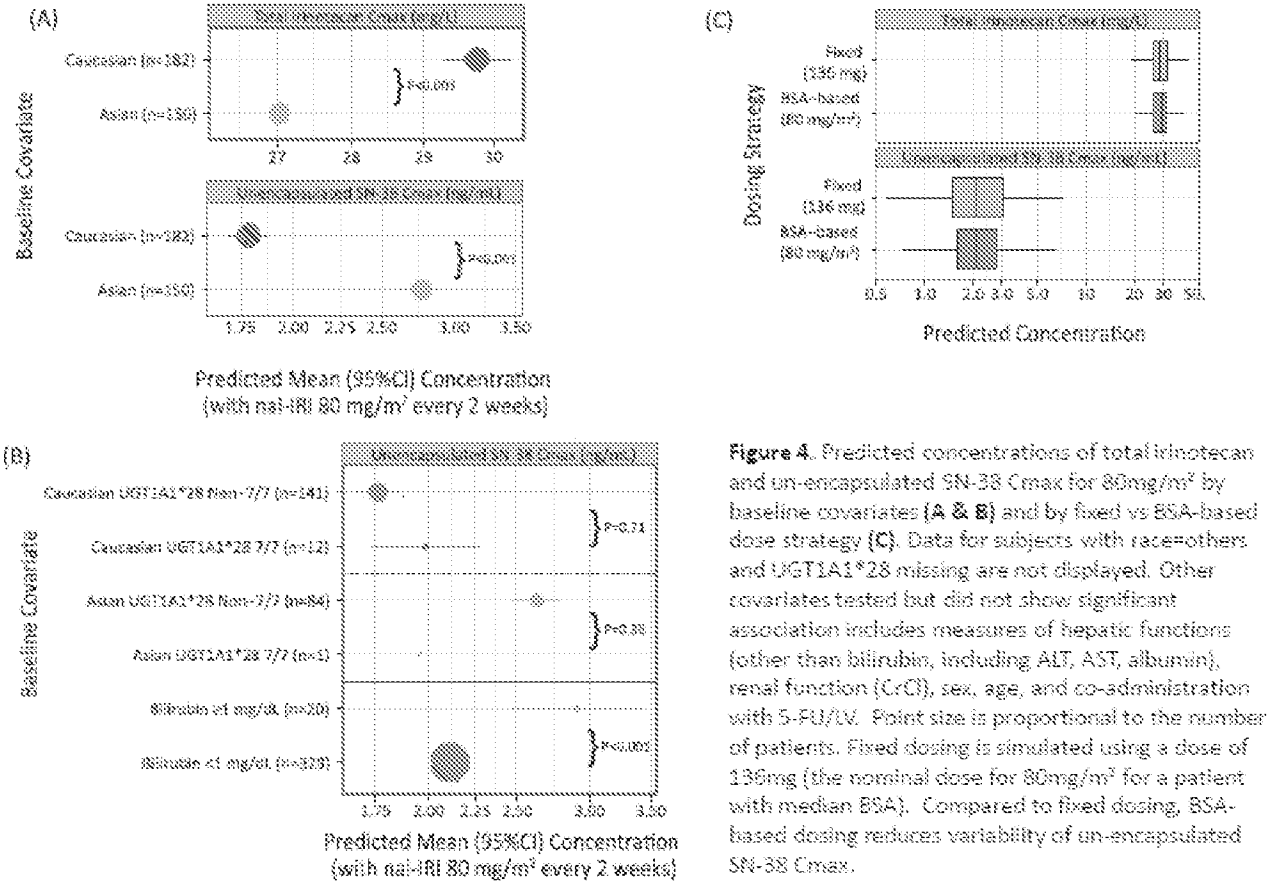

Figure 4. Predicted concentrations of total irinotecan and un-encapsulated SN-38 Cmax for 80mg/m^2 by baseline covariates **(A & B)** and by fixed vs BSA-based dose strategy **(C)**. Data for subjects with race=others and UGT1A1*28 missing are not displayed. Other covariates tested but did not show significant association includes measures of hepatic functions (other than bilirubin, including ALT, AST, albumin), renal function (CrCl), sex, age, and co-administration with 5-FU/LV. Point size is proportional to the number of patients. Fixed dosing is simulated using a dose of 136mg (the nominal dose for 80mg/m^2 for a patient with median BSA). Compared to fixed dosing, BSA-based dosing reduces variability of un-encapsulated SN-38 Cmax.

PK-Efficacy Association

In the nal-IRI+5-FU/LV arm of NAPOLI-1, longer OS and PFS were associated with higher Cavg of tIRI, tSN38, and uSN38, with the highest association observed for both tSN38 and uSN38. The relationship between OS and quartiles of uSN38 is provided in **Figure 5**. Sensitivity analysis using adjustment for dose modification showed that the association between higher exposure and longer survival was maintained. Higher exposures were associated with higher probability of achieving objective response (OR) in the nal-IRI+5FU/LV arm (**Figure 6**). The association was not observed in the nal-IRI monotherapy arm.

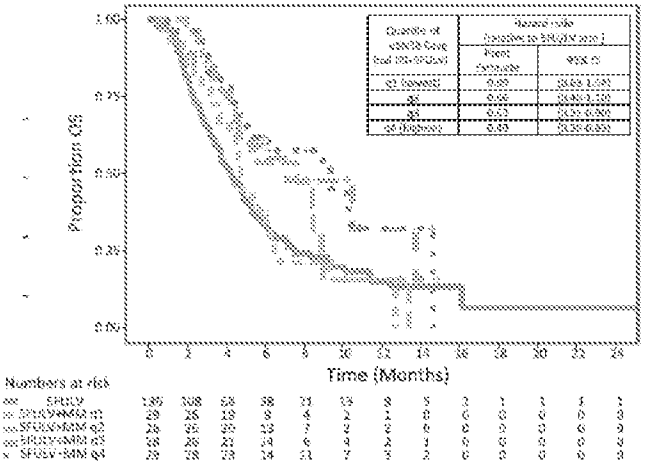

Figure 5. Kaplan-Meyer Plot of Overall Survival (OS) by Quartiles of Un-encapsulated SN-38 Average Concentration (Cavg) in the nal-IRI+5-FU/LV arm of Study.

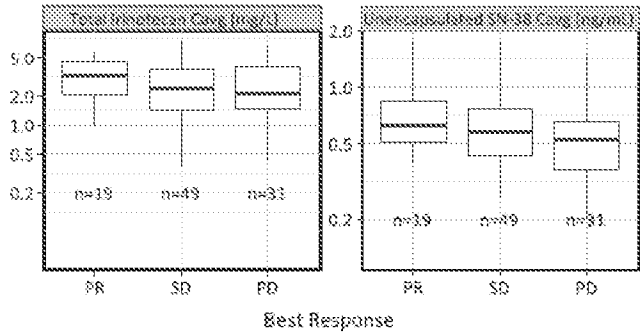

Figure 6. Association between Best Objective Response and average concentrations of total irinotecan and un-encapsulated SN-38 in the nal-IRI+5-FU/LV arm of NAPOLI-1. PR= partial response, SD= stable disease, PD=progressive disease.

PK-Safety Association

In the dataset of 353 patients, neutropenia is associated with uSN38 Cmax (**Figure 7**). The association with neutropenia was stronger for uSN38 Cmax than for tSN38 Cmax. Univariate analysis showed that uSN38 Cmax is associated with neutropenia, after adjusting for baseline absolute neutrophil count and co-administration with 5FU/LV (two known factors associated with neutropenia).

In the same dataset, diarrhea was associated with tIRI Cmax (**Figure 8**). The tIRI Cmax was observed at higher values for the nal-IRI monotherapy arm (120 mg/m^2 every 3 weeks) than for the nal-IRI+5FU/LV arm (80 mg/m^2 every 2 weeks) because of the difference in nal-IRI doses. The association was observed within the nal-IRI monotherapy arm, but not within the nal-IRI+5FU/LV arm; this is likely due to the higher tIRI Cmax values observed in the nal-IRI monotherapy arm than those in the nal-IRI+5FU/LV. Multivariate analysis showed that tIRI is associated with diarrhea in each of the Caucasian and Asian subgroups.

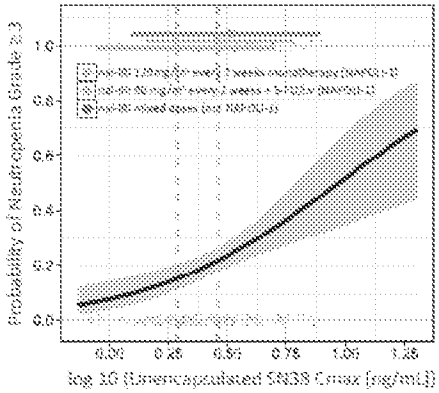

Figure 7. Incidence rates of neutropenia grade ≥3 by un-encapsulated SN-38 Cmax in patients treated with nal-IRI. Small points=concentrations per patient with and without neutropenia. Large points (range)=median (95th% percentiles) of each dose group.

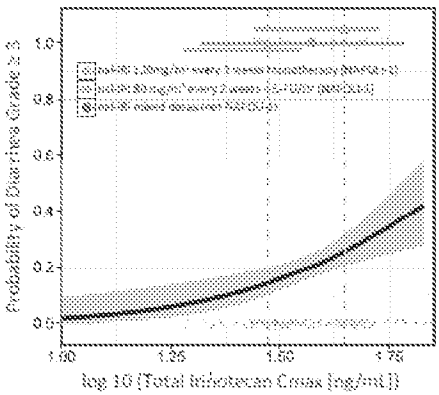

Figure 8. Incidence rates of diarrhea grade ≥3 by total irinotecan Cmax in patients treated with nal-IRI. Small points=concentrations per patient with and without diarrhea. Large points (range)=median (95th% percentiles) of each dose group.

Conclusions

1. A mechanism-based population plasma PK analysis was developed for nal-IRI.
2. Un-encapsulated SN-38 Cmax is associated with neutropenia and is influenced by BSA, race, and bilirubin; total irinotecan Cmax is associated with diarrhea and is influenced by race.
3. In patients with metastatic pancreatic cancer previously treated with gemcitabine-based therapy (NAPOLI-1), higher total irinotecan and SN-38 plasma concentrations are associated with longer OS and PFS, and greater OR.
4. The population PK modeling shows that the nanoliposomal formulation of irinotecan (nal-IRI) confers a superior PK (lower uSN38 Cmax and longer half life) than irinotecan HCl, while exerting significant anticancer benefits.

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Nanoliposomal irinotecan (nal-IRI, nal-IRI) population pharmacokinetics (PK) and its association with efficacy and safety in patients with solid tumors

Poster 327
Abstract 2965

Baxalta

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Introduction

Results

Pharmacokinetic Analysis

Safety

Conclusions

References

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European Cancer Congress 2015 (ECC 2015), Vienna, Austria

Biomed. & Pharmacother., 43 (1989) 237-250

Dossier

Oxalato-platinum or l-OHP, a third-generation platinum complex : an experimental and clinical appraisal and preliminary comparison with cis-platinum and carboplatinum

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Summary — A new platinum complex, oxalatoplatin or l-OHP, which, at the same metal dose in experimental tests is as efficient as cisplatin, and is more so at a lower metal dose than carboplatin; which is as efficient in human tumors of the testis and ovary as these other analogs, and more so in melanoma and breast cancer; which is not nephrotoxic, cardiotoxic or mutagenic, and hardly hematotoxic and neurotoxic, is described and compared with the above-mentioned platinum complexes.

Combined with 5Fu, it induces a high number of remissions in colorectal cancer, and has brought about cures in inoperable gastric cancers. Combined with carboplatin, it has resulted in a high proportion of cures in L1210-carrying mice, which no other two-by-two combination of these complexes has achieved.

l-OHP / cis-platinum / carboplatin

Résumé — L'oxalatoplatine ou l-OHP, une troisième génération de complexe de platine, bilan expérimental et clinique actuel et comparaison préliminaire avec le cis-platine et le carboplatine. *Un nouveau complexe de platine, l'oxalatoplatine ou l-OHP, qui, pour la même dose de métal, s'est avéré aussi efficace dans les tests expérimentaux que le cis-platine et davantage pour une dose de ce métal plus faible, que le carboplatine; qui est aussi efficace contre les tumeurs humaines du testicule et de l'ovaire que ces deux analogues-là, et davantage sur le mélanome et sur le cancer du sein; qui n'est ni néphrotoxique, ni cardiotoxique, ni mutagénique, et qui est à peine hématotoxique et neurotoxique, est décrit et comparé avec les complexes de platine ci-dessus.*

Combiné avec le 5Fu, il a induit un nombre élevé de rémissions dans le cancer colorectal et a induit quelques guérisons dans des cas de cancers gastriques inopérables. Combiné avec le carboplatine, il permet d'obtenir une haute proportion de guérisons de souris porteuses de leucémie L1210, qu'aucune autre combinaison des sels de platine deux à deux ne permet d'obtenir.

l-OHP / cis-platine / carboplatine

* *Correspondence and reprints.*

Introduction

Since Rosenberg [37] described the oncostatic effect of cisplatin (cis-$PtCl_2$ $(NH_3)_2$, or CDDP), the number of complexes of this metal [30] has increased much more than the precise knowledge of the mechanisms behind their cytostatic effect, which mainly consist of reactions with nucleophilic sites of DNA, causing intrastrand and interstrand crosslinks (Fig. 1), as well as DNA-protein crosslinks [28].

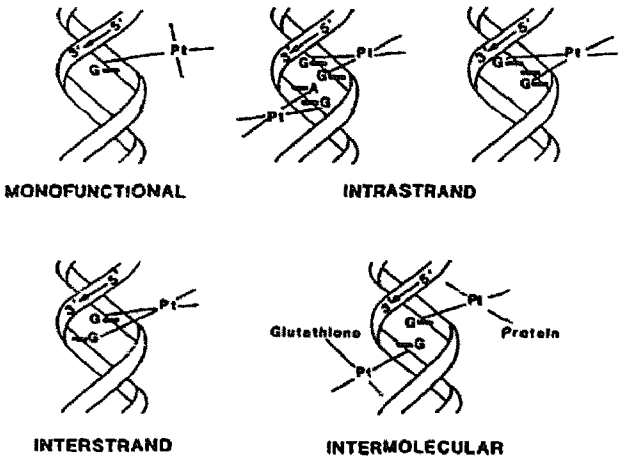

Fig. 1. Structures of the various adducts produced in DNA by afinum complexes [30].

Cisplatin indeed represents major progress in clinical cancer chemotherapy, as its oncostatic potential has been made positive use of, especially in testicular and ovarian carcinomas [43].

Unfortunately it is a rather toxic drug and has two major short-term side-effects : vomiting [43] and kidney lesions [43]. It is also a mutagenic agent [11].

Among the second-generation platinum complexes (Fig. 2; Table I), we have studied CHIP (or cis-dichloro-trans-dihydroxy-bis (isopropylamine) platinum IV) [35], which appeared to be as

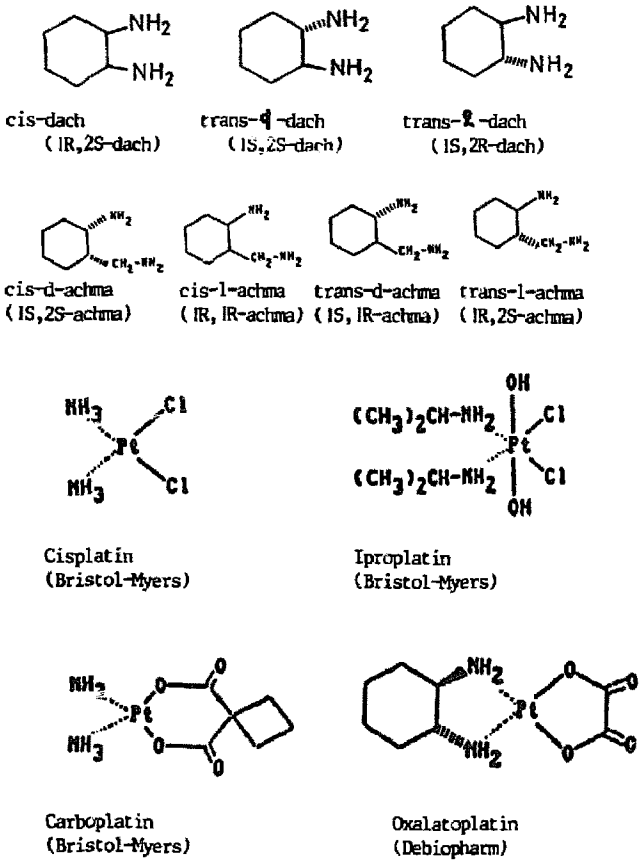

Fig. 2. Principles of platinum complex structures, cisplatin and the second and third generation complexes.

Table I. Second-generation platinum complexes.

Internationally recognised name	*Laboratory of origin*	*Abbreviation*	*Chemical name (m.w., water solubility)*
Cisplatin	Bristol-Myers	CDDP NSC 119875	Cis-diammino-dichloro-platinum (II) m.w. = 300.1 w. sol. = 1 mg/ml
Carboplatin	Bristol-Myers	CBDCA NSC 241240	Cis-diammin(cyclobutane-dicarboxylato-1, 1(2-0)-0,0) platinum (II) m.w. = 371.1 w. sol. = 17 mg/ml
Oxalatoplatin	Debiopharm Roger Bellon Rhône Poulenc	l-OHP ICIG 2036	Oxalato (1R, 2R-cyclohexane-diammine) platinum (II) m.w. = 397.1 w. sol. = 7.9 mg/ml

efficient as CDDP and no more toxic. CBDCA or carboplatin (or cis-diammino-1, 1-cyclobutane dicarboxylato platinum II) (in which the bidentate dicarboxylate chelate ligand replaces the 2 chlorine atoms of cisplatin) has however, found preference; it induces the same molecular lesions as the latter in L1210 cells, but is much less active (45 times less) as it is much slower to induce DNA-interstrand and DNA-protein crosslinks [28]. Thus a higher dose of the metal platinum has to be applied than with CDDP to obtain the same effect. Experimental tumors, expecially L1210 leukemia, which are resistant to cisplatin are also resistant to carboplatin [42]. CBDCA causes less toxicity for the kidney than CDDP [3], which may increase anthracyclin toxicity in the case of their combination.

Hence we have concentrated our efforts on the search for non-nephro-toxic, non-mutagenic, less hemato- and cardiotoxic and, of course, more active platinum complexes.

In the "carrier ligand-Pt-leaving group", the group dach (1,2-cyclohexamediamine) (Fig. 2) appeared to be one of the most active ligands. As we had previously studied malonate 1R, 2R-dach Pt (IV) complex [1], which was very active but unfortunately not water-soluble enough to be used in humans, we focussed our interest on oxalate 1R, 2R-dach Pt (IV), being l-OHP, which appeared to us to be as or more experimentally effective than CDDP and much less toxic.

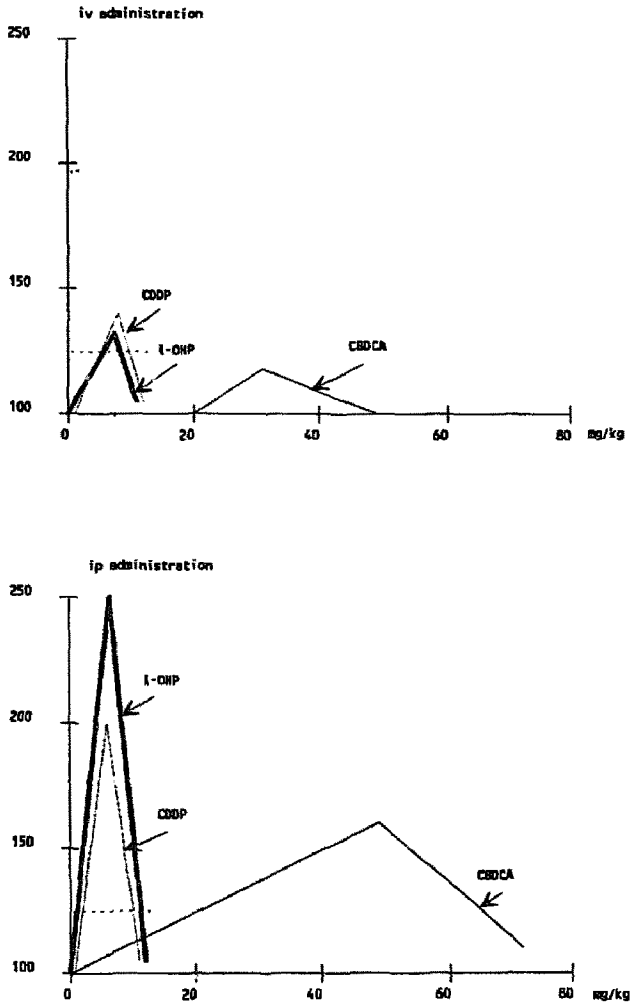

Fig. 3. Comparative curves of the maximally efficient dose ranges (MEDR) of the 3 studied platinum complexes [24].

Murine tumors

Leukemia-lymphomas

The cytostatic effects of l-OHP on L1210 leukemia expressed by the MEDR (maximally efficient dose range) appeared to us [24] to be equal to that of CDDP and higher than that of CBDCA applied at a much higher dose (Fig. 3). The i.p. administration for the 3 complexes is significantly more active than the i.v. (Fig. 3).

l-OHP is of particular interest as it works on the T-leukemia-lymphoma L40 AKR [24] and on the B large-cell lymphoma LGC [24], while CDDP has no effect on the latter [24] (Table II). According to Tashiro [41], l-OHP works as well as CDDP on the histiocytic sarcoma M5076.

Brain neoplasias

l-OHP is active on brain injected L1210 leukemia, while CDDP is not (Table II) [24].

Table II. a) Effects of l-OHP and CDDP on L40 AkR grafted leukemia and LGC lymphoma.

	mg/kg i.v.	*I*[a]	
		l-OHP	*CDDP*
L40 AkR leukemia	5	144	194
	7.5	177	Toxic
LGC lymphoma	5	∞[c]	NA
	7.5	NA[b]	NA

Tumor graft (10^6 cells, i.p.) on day 0.
Treatment i.v. on days 1, 5 and 9.
[a] $I = T/C \times 100$.
[b] NA, not active.
[c] ∞, more than 50% of mice were cured.

Table II. b) Comparison of the effect of l-OHP and CDDP on intracerebrally grafted L1210 leukemia (10^4 cells) [24].

	I^a	P^b
l-OHP	165	0.02
CDDP	100	

Dose 5 mg/kg i.p.
[a] I = T/C × 100.
[b] P, statistical significance.

Solid tumors
Table II summarizes the results with l-OHP on different murine solid tumors studied by us [24] and by Tashiro [41].

Test for efficiency of adjuvant therapy
We have submitted the mammary tumor MA16c (which carries sex hormone receptors) to l-OHP as post-surgical adjuvant therapy; this platinum complex is efficient, as it cured >43 % of mice (Table IV) [24].

Cross-resistance
All tumor lines studied by Saijo *et al.* [39] and resistant to CDDP are also cross-resistant to CBDCA. On the contrary, l-OHP is, according to Kidani [19], active on the CDDP resistant L1210 leukemia (Fig. 4).

5-Fluorouracil (5-Fu) potentiation and cross-synergism
Figure 5 shows the dose variable, but considerable synergistic action between 5Fu (modulated by folinic acid) and l-OHP [22].

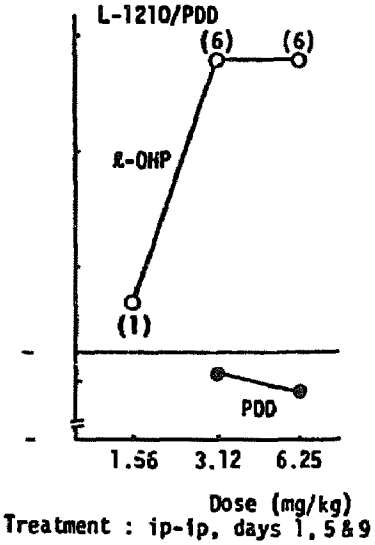

Fig. 4. l-OHP is active on a CDDP-resistant L1210 leukemia [19].

Table IV. Percentage cured animals at day 60 after CT, HT, IT and surgery alone [24].

	Treatment alone	*Neo-adjuvant*	*Adjuvant*	*Neoadjuvant + adjuvant*
CT	40	62	66	43
HT	10	55	40	50
IT	0	0	6	21
Surgery	0			

CT = Chemotherapy : *l*-OHP 5 mg/kg i.p., days 1, 5, 9 neoadjuvant (N); days 21, 25, 29 adjuvant (A), or days 1, 5, 9, 21, 25, 29 (N + A).
IT = Immunotherapy : Zinc gluconate : 6 mg/kg p.o. + bestatin 6 mg/kg p.o. days 1—21 for N, 21—42 for A, or 1—42 (N + A).
HT = Hormonotherapy : D-Trp-6-LH-RH 100 µg/kg i.p. days 1—21 for N, 21—42 for A, or 1—42 (N + A).

Table III. Experimental antitumor activity [24, 41].

Reference screening center	*Tumor graft criteria of evaluation*	*Treatment schedule*	*Daily dose range (mg/kg)*	*Optimal T/C (%)*	*Drugs compared*	*Daily dose range (mg/kg)*	*Optimal T/C (%)*
24	MA 16-C	1, 5, 9	7.5—5.0	206	CDDP	5	inactive
					CBDCA	50	inactive
41	B_{16} melanoma sc survival	1, 5, 9 i.p.	10—2.5	128	CDDP	10—2.5	139
					CBDCA	12.5—2.5	170
41	Lewis lung sc survival	q2d, d1—19 i.p.	5—1.25	159	CDDP	2.5—1.25	184
					CBDCA	60	245
41	C_{26} colon carcinoma survival	1.5 i.p.	12.5—3.12	143	CDDP	12.5—1.56	322
					CBDCA	25	

l—OHP = oxalatoplatin; CDDP = cisplatin; CBDCA = carboplatin.

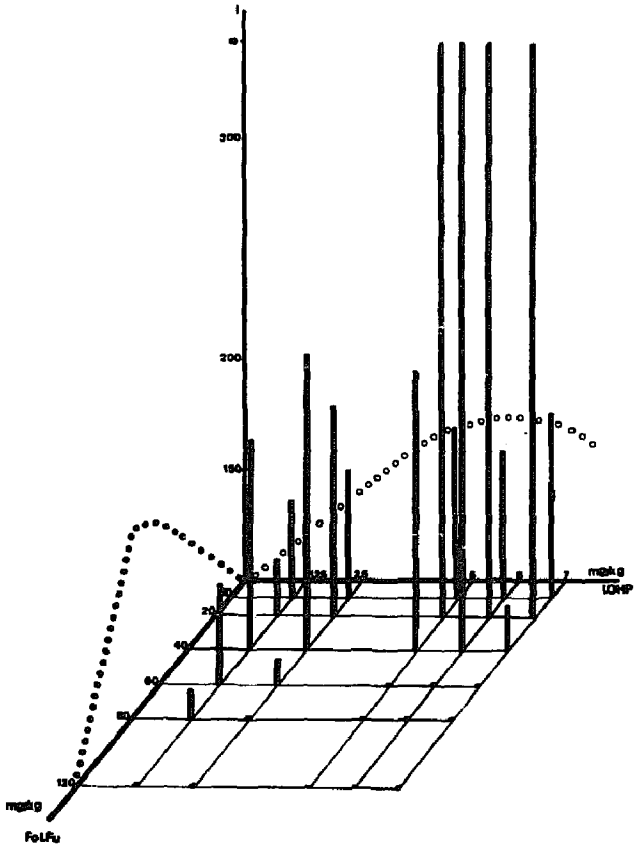

Fig. 5. Synergism between fluorouracil (modulated by folinic acid) and l-OHP studied on L1210 leukemia [22].

Table V. Simultaneous association of the two platinum complexes in the therapy of L1210 leukemia (unpublished).

Platinum complexes	*Dose (mg/kg/d)*	*Injection (day)*	*I*
Half optimal doses			
CBDCA	25		
+		1,5,9	200
l-OHP	3		
CBDCA	25		
+		1,5,9	233
CDDP	3		
CDDP	3		
+		1,5,9	188
l-OHP	3		
Optimal doses			
CBDCA	50		
+		1,5,9	100 Tox.
CDDP	6		
CBDCA	50		
+		1,5,9	∞
l-OHP	6		
CDDP	6		
+		1,5,9	100 Tox.
l-OHP	6		

Still more interesting is the effect of the exclusive combination of l-OHP and carboplatin, as it cured 70 % of the L1210-carrying mice, *versus* no cure with either compound administered alone, and no cure by any other possible two-by-two combinations of the 3 compounds studied (Table V) [22].

Comparative species toxicity

Table VI shows the non-toxicity of l-OHP registered with the 3 doses of the MEDR in mice and with those extrapolated to baboons and humans. This contrasts with CDDP which is highly toxic for the kidney (Table VII), and with carboplatin, which is less toxic for the kidney than CDDP, but more toxic for hemopoiesis (Table VIII). In electron microscopical study, we have observed cardiac toxicity [3] for CDDP but not for l-OHP.

CDDP [11] and carboplatin [6] are mutagenic by the conventional test, while l-OHP is not [41] (Table IX).

Phase I studies and human pharmacokinetics

The phase I trials have indicated that the dose for phase II trials was 100 mg per cycle every 3 weeks for CDDP, the dose limiting factor being renal, hematopoietic and neurotoxic [20], 400 mg per cycle every 3 weeks for carboplatin [42], the limiting dose factor being hematopoietic [7, 15, 27], and 100 mg per cycle every 3 weeks for l-OHP (Tables X and XI) [23], the only dose-limiting factor being neurotoxicity, which Marty [14] induced by increasing the cycle dose to 200 mg/m^2, well above our recommendations.

Comparative pharmacokinetics of CDDP, CBDCA and l-OHP are given in Tables XII and XIII and in Figure 6 [33].

Phase II trials in human tumors

The object of our phase I trails, which use the intra-patient escalation method rather than the

Table VI. I-OHP : Grading of histologic toxicity in mice and baboon, and of clinico-biologic toxicity in man. Doses indicated by the MEDR in mice : 45—56—67 mg/m² [24, 25].

Species	Dose (mg/m²)	Hemopoiesis				Liver			Kidney		Heart (μ M)
		Hb	WBC	PMN	Plat.	SGOT	SGPT	Al.Ph.	Urea	Creat.	
Mice	45		0				0		0	0	0
	56		0				0		0	0	0
	67		0				0		0	0	0
Baboon	45	0	0	0	0	0	0	0	1	0	0
	56	0	0	0	0	0	0	0	1	0	0
	67	0	0	0	0	0	0	0	0	0	0
											Cardio-echography
Human	45	0	0	0	0		0	0	0	0	0
	56	0	0	0	0		0	0	0	0	0
	67	0	0	0	0		0	0	0	0	0

Table VII. CDDP : Grading of histologic toxicity in mice and baboon, and of clinico-biologic toxicity in man. Doses indicated by the MEDR in mice : 45—56—67 mg/m² [24].

Species	Dose (mg/m²)	No. of doses	Hemopoiesis			Liver		Kidney	
			WBC	PMN	Plat.	SGOT	SGPT	Urea	Creat.
Mice	45								
	56			1		0		2	3
	67								
Baboon	57	1	0	2	0	0		1	0
		3	1	2	3	0		3	2
Human	4	1			0				0
	56	1		0(0—3)	0				⩾2
	67	1		1(0—3)	0				>2
	100	1		0(0—3)	0				>2

Table VIII. CBDCA : Grading of histologic toxicity in mice and dog, and of clinico-biologic toxicity in man. Doses indicated by the MEDR in mice : 360—450—540 mg/m² [10]*.

Species	Dose (mg/m²)	No. of doses	Hemopoiesis				Liver			Kidney	
			Hb	WBC	PMN	Plat.	SGOT	SGPT	Al.Ph.	Urea	Creat.
Mice	360			3		ND		ND			ND
Dog's	400			2		2		ND			ND
Human	200	7		3		4					
(minimum	300	5		0		1		ND			ND
nadir)	350	4		1		0					
	400	6		1		0					
	500	5		4		3					
	520	5		3		4					

ND : Not determined.

* The data presented by Carter and Hellman are incomplete in mice and large animals.

Table IX. Non-mutagenicity of Pt oxalato DACH isomers for *S. typhimurium* [19]*.

Pt complex		*Revertant/plate*				*Mutagenicity*
		TA 100		*TA 98*		
Isomer	*μg/plate*	*+ S-9 mix*	*— S-9 mix*	*+ S-9 mix*	*— S-9 mix*	
Trans-l	1	93	70	23	13	(—)
(l-OHP)	5	46	57	22	18	
	10	46	57	25	25	
	50	12	13	10	7	
Trans-d	1	74	93	36	14	(—)
	5	52	76	28	20	
	10	36	50	12	8	
	50	0	0	0	0	
Cis	1	60	71	28	15	(—)
	5	80	68	27	26	
	10	52	60	36	28	
	50	13	22	5	14	
Control		74	75	27	16	

* Mutagenicity of l-OHP for *S. typhimurium* strains TA 100 and TA 98 was determined together with its stereo isomers. None of them were mutagenic for both *Salmonella* strains.

Table X. Phase I study of l-OHP (intra-patient dose escalation method) : toxicities [25].

Dose Level	*No. patients*	*Toxicity*							
		Nausea, vomiting	*Lung*	*Heart*	*Liver*	*Kidney*	*Hematopoiesis*		
	N = 23						*Hb*	*WBC*	*Platelet*
0.45	21	—	—	—	—	—	—	—	—
4.5	21	—	—	—	—	—	—	—	—
9	9	—	—	—	—	—	—	—	—
15	12	—	—	—	—	—	—	—	—
22.5	8	—	—	—	—	—	—	—	—
30	9	1/9	—	—	—	—	—	—	—
45	19	19/19	—	—	—	—	1/19 Gr1	—	—
56	15	15/15	—	—	1/15	—	1/15 Gr1	—	—
67	11	11/11	—	—	—	—	1/11 Gr2	—	1/11 Gr1
100	25	60 %					4 %	4 %	4 % [23]

Parameters evaluated :
Liver : transaminases, alkaline phosphatase.
Kidney : area, creatinine.
Cr : grade according to WHO [8].

Table XI. Phase I study of l-OHP (intra-patient dose escalation method) : anti-tumor activity for the cycle maximum doses ⩽ 67 mg, for 100 mg [25].

Response	*No. patients*	*Tumor + target*	*Total dose received*	*Imaging*
Progressive disease	16/23			
		1 prostate + liver and bone metastasis	798 mg	Echo + PAP
Stabilisation	3/23	2 liver	843 mg	
		3 liver	943 mg	αFP
Minor response	1/23	Lung	740 mg	Tomo-scan
Partial response	1/23	Breast carcinoma + bone metastasis	473 mg	Scintigraphy
Complete response	1/23	Melanoma + metastases of the lung and parotid	297 mg*	Scan (of the head and lung)

* *NB* : This patient is still under study. Although he has reached only a low level (45 mg/m²) at the time of this report, the results as evidenced by scan of the and the head confirmed a complete disappearance of the metastases of the lung and the parotid seen before treatment.

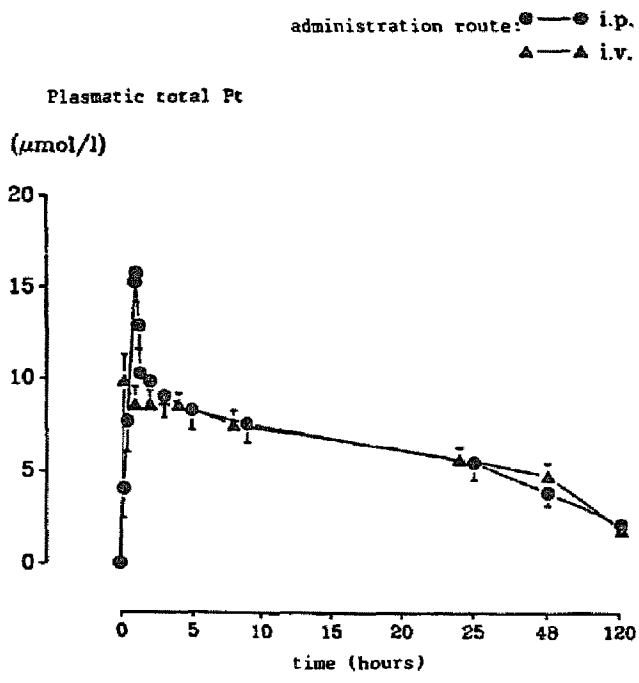

Fig. 6. Comparison of the i.v. and i.p. routes after administration [34].

conventional interpatient escalation type, considered unethical by our Committee of Ethics, Methodology and Economics [4], is to find not the maximally tolerated dose but the active dose, which is lower than the former for most drugs we have studied [26].

Table XII. Compared pharmacokinetic parameters of cisplatin, carboplatin and oxalatoplatin [33].

Pharmacokinetic parameters	*Cis-platin*	*Carbo-platin*	*Oxalato-platin*
$T^{1/2}$ α total Pt (min.)	8.7—912	10.8—98	ND
$T^{1/2}$ β total Pt (h.)	30.5—290.4	16.6—98	70.1
$T^{1/2}$ α free Pt (min.)	2.7—78	5.7—125	ND
$T^{1/2}$ β free Pt (min.)	25.9—226.8	102—436	ND
% Bound protein 4 h. post—infusion	90	24	ND
Excretion units/24 h. (% of dose admin.)			ND
Renal clearance of free Pt/FG count	0.38—3.62	0.7	ND
Plasma clearance of total Pt (ml/min/m²)			5.9
Plasma clearance of free Pt (ml/min/m²)	15.6—658	40—123	ND
Vol. (ml/m²)	52.3—65.6	16.1—24	36.5
total Pt	21.2—50.5	17.3	
free Pt	23.6—81.9	16	
$T^{1/2}$ free Pt *in vitro* (h)	1.5—3.7	30	
C_{max} total Pt plasm. (μmol/l)			16.6
T_{max} total Pt (h)			1
AUC (μmol/l·h)			749.4
MRT (h)			102.5
Biliary excretion (% dose admin.)	< 0.06		

Having observed that the murine MEDR extrapolated to man is perfectly tolerated, we have conducted the first phase II trials by continuing to escalate the dose of the phase I trial [23] without reaching toxic amounts, and have chosen 100 mg per cycle.

Table XIII. Compared pharmacokinetic parameters of carboplatin, cisplatin and oxalatoplatin (by peritoneal administration) [33].

Pharmacokinetic parameters	*Cis-platin*	*Carbo-platin*	
$T^{1/2}$ β of elimination (h)			Mathé 61.8 Fredj 68.8 NS*
Clearance of plasm. of total Pl (ml/min·m²)		16.7	M 7.5 F 6.4 NS*
Renal clearance of free Pt (ml/min·m²)	86—126		
Peritoneal clearance of total Pl (ml/min/m²)		11.3	
Peritoneal clearance of free Pl (ml/min/m²)	43.3	7	
Excretion units·24 h (%) dose admin.)	23—37	64	
Vol. (l/m²)			M 40.3 F 30.6 NS*
C_{max} total Pl plasm. (μmol/l)	50—360	10—30	M 7.35 F 8.9 S*
C_{max} free Pl plasm. (μmol/l)	150—280		
Peritoneal C_{max} (μmol/l)		500	
Peritoneal T_{max} (h)			M 1.25 F 4.65 S*
Peritoneal MRT (h)	0.85	4.7	M 91.8 F 100.7 NS*
AUC (μmol/l·h)			M 553.6 F 857.4 NS*
Peritoneal (4 h)		908	
Plasmatic		50	
R = perit./plasm.	12.4	18.2	
Peritoneal (24 h)		1107	
Plasmatic		173	
R = perit./plasm.		6.2	

* Wilcoxon test : S = $p \leq 0.05$ significant difference from i.v.
NS = no significant difference between i.p. and i.v.

Platinum complexes applied as single drugs. The preliminary results of our l-OHP phase II trial are very promising in testicular and ovarian cancers, non-Hodgkin's lymphomas, gliomas, head and neck tumors, and lung small-cell carcinoma.

We shall only compare here the results for melanoma, ovary and breast cancer, for which we have sufficient patients (Table XIV). The same proportion of responses (complete + "partial > 50 %" remissions) is registered in ovarian cancer previously treated with CDDP, CBDCA or l-OHP (≈ 30 %). We have not conducted phase II trials of l-OHP applied as a single drug in not previously treated patients, as other authors have done with CDDP and CBDCA, since in our opinion, this is highly unethical [4].

In melanoma, we have observed a 33 % response (including complete remissions). l-OHP is the only drug to achieve such remissions and at this incidence.

In breast cancers, we have not obtained more remissions with the usual bolus administration than other authors with CDDP. But by administering l-OHP according to the chronopharmacological modality, we have registered a 20 % response rate (Table XIV).

Combinations of platinum complexes with 5-fluorouracil. These combinations have been studied in colorectal cancer. If we consider the application in a 3—5-day cycle, which is the usual one, one registers between 0 [29] and 25 % [5, 40] for CDDP, between 12 % [18] and 40 % [16] for carboplatin. For l-OHP, if we combine 5-fluorouracil modulated with folinic acid [21], our response rate is 23.5 % for bolus injection, and 60 % when we apply it according to the chronobiologic modality (Table XV).

In gastric cancer, we have not found combinations reduced to 5Fu and CDDP or carboplatin; combined with 5Fu, l-OHP has given us 5 responses out of 8 patients, among which there were 2 complete tumor disappearances in non-operable patients who were checked microscopically at second-look surgical intervention (Figs. 7, 8) (unpublished results).

Dose-related nausea and/or vomiting occur in many patients receiving cisplatin (≈ 80 %) and carboplatin (≈ 43 %) [2, 8]. In the latter case it was noted that vomiting was delayed 6—12 h after administration of the drug. The incidence of these side-effects is 60 % in the case of l-OHP (Table XVI).

As far as severe side-effects are concerned, Table XVI shows that l-OHP has never been as clinically nephrotoxic as CDDP is [30b], and that

it is much less frequently hematoxic than CBDCA [9].

At a dose of < 100 mg per cycle, i-OHP is still less frequently ototoxic than cisplatin, but it induces paresthesias more often than the other two platinum complexes : these initial signs of neurotoxicity are in fact useful to indicate the risk of serious manifestations, and prompt an interruption of the treatment before the latter appear.

Table XIV. Efficacity of *l*-OHP *versus* CDDP—CBDCA.

	Melanoma (Previously and not previously treated) (%)	*Ovary*		*Breast Previously treated) (%)*
		Not previously treated (%)	*Previously treated (%)*	
CDDP	10 (a)	57.6 % (b,c,d)	25 (b,c,d)	6 (e)
CBDCA	11.5 (f—g) (4—19)	59 (h)	23 (i)	0 (j)
1-OHP	33	—	28	0 (bolus) 20 (chrono)*

* Pharmacological modality.
(a) Al-Sarraf M. (1982) Cisplatin hydration with and without mannitol diuresis in refactory disseminated malignant melanoma : A. Southwest Oncology Group Study (8b).
(b) Niijima T. *et al.* : Gan to Kagakuryoho 9(1), 46-54 (30b).
(c) Kawai H. *et al.* : Gan to Kagakuryoho 9(3), 433-442 (30b).
(d) Kato T. *et al.* : Gan to Kagakuryoho 9(4), 694-701 (30b).
(e) Ostrow S. *et al.* (1980) High-dose cis-diamminedichloro-platinum therapy in patients with advanced breast cancer : pharmacokinetics, toxicity and therapeutics (8b).
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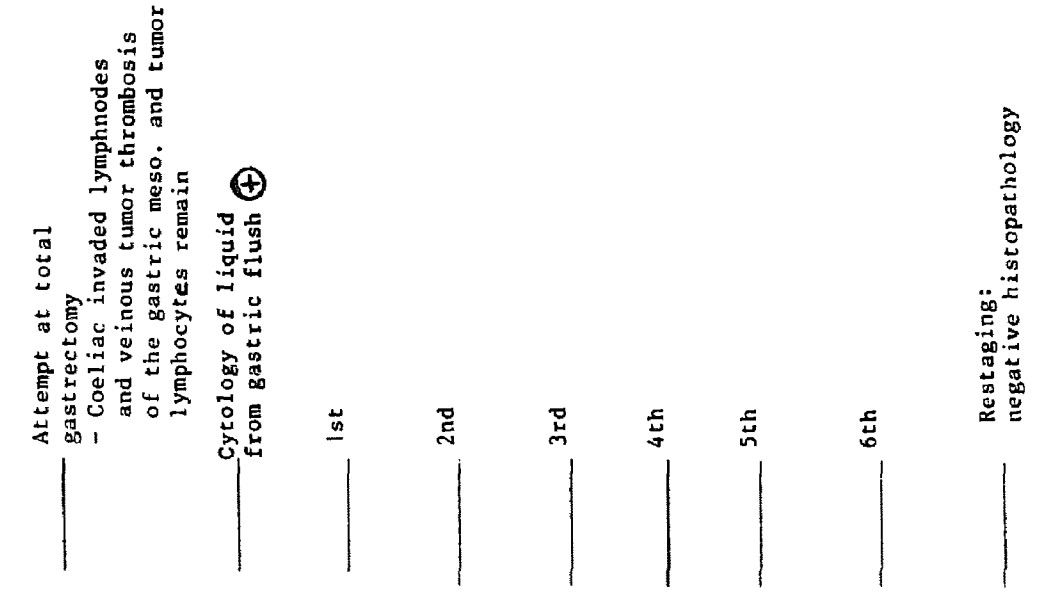
(g) Evans L.M. *et al.* (1987) Phase II trial of carboplatin in advanced malignant melanoma (8b).
(h) Swenerton K.D. (1986) The efficacy and toxicity of carboplatin in previously treated patients with advanced ovarian cancer (8b).
(i) Booth B.W; *et al.* (1985) Phase II trial of carboplatin in advanced breast carcinoma : a cancer and leukemia B. study (8b).
(j) Canetta R.M. *et al.* (1984) Developing new drugs for ovarian cancer : a challenging task in changing reality (8b).
We give as the references a, b, c etc. those articles in which the proportion published corresponds to that of the general means of all the trials.

Table XV. *l*-OHP combination phase II trial.

	Protocol	*Modality*	*PTS*	*CR*	*PR*	*% Response*	*SD*	*PD*
Rectocolon	+ 5-FU folinic acid	Bolus	34	1	7	23.5	19	7
	+ 5-FU follonic acid	Chronotherapy	31 (20*)	—	13 (12*)	42 (60*)	14 (7*)	4 (1*)
Stomach	+ 5-FU	Bolus	8	2	3			

* Among whom 20 had not been previously treated by 5FU chrono-12 PR (60 %).

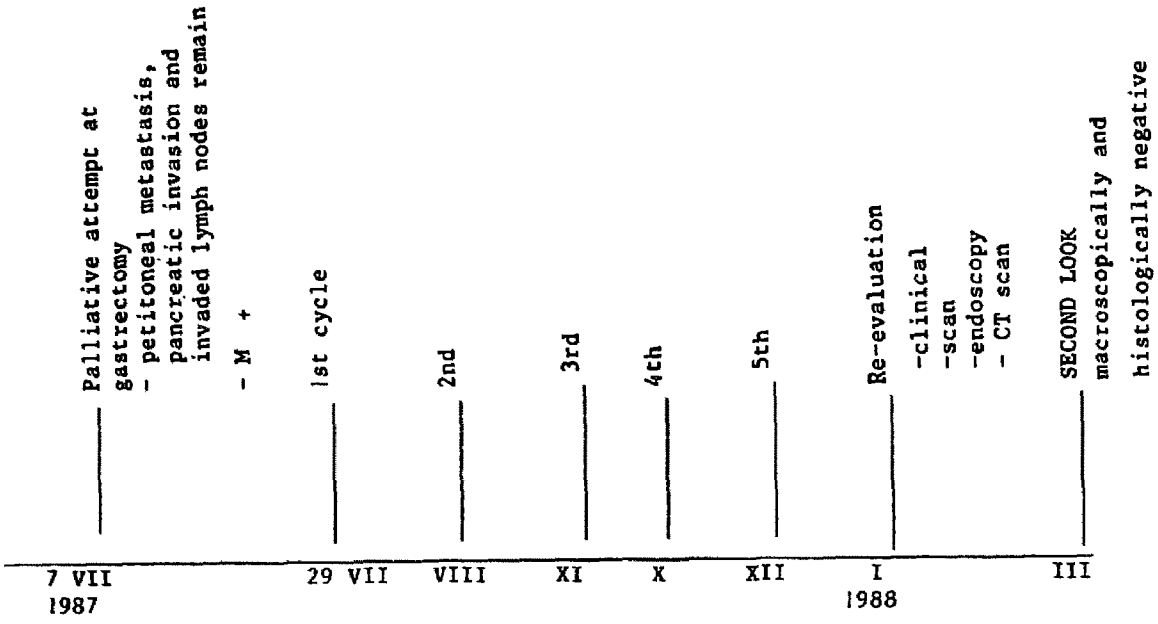


	20 VI 1988	VII	VII	VIII	IX	X	XI	XII	I 1989
Ag CA 50		60.9		17.9	14.9		7.4		
LASA		29.7		11.5	11.3		9.1		
Day 1 l-OHPmg			100	100	90	100	110	110	
Day 1-5 5FUmg			1000	1000	1000	1000	1000	1000	
i.v.			600	600	600	600	600	600	
i.p.			400	400	400	400	400	400	

Fig. 7. Treatment of an inoperable gastric cancer in a 40-yr old male with l-OHP combined with 5Fu : complete remission.

Table XVI. Toxicity of LOHP *versus* CBDCA and CDDP.

400 mg/m²/4 Wk	*l-OHP (%)*	*CDDP [30b] (%)*	*CBDCA [9] (%)*
		100 mg/m²/3—4 Wk	
Myelosuppression (> GR II WHO)			
leucopenia	4	31.7	55
thrombocytopenia	4	21	32
anemia	4	28	59
Nephrotoxicity (> GR II WHO)			
creatinin serum	0	9	7
Gastrointestinal (> GR II WHO)			
vomiting	60	78	53
diarrhoea	0	8.6	6
Neurotoxicity—GR III WHO	0	22	2
—GR II WHO	28		
Ototoxicity (> GR II WHO)	0	3.7	1.1
mucositis	0	0.6	2
alopecia	0	2.9	2



Day 1 1-OHP mg	100	100	100	110	120
Days 1-5 5Fu	1000	1000	1000	1200	1300
CEA	25.6		1.1	1.4	

Fig. 8. Treatment of an inoperable gastric cancer in a 43-yr old male with l-OHP combined with 5Fu : complete remission.

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Randomized phase II trial of S-1 versus S-1 plus irinotecan (IRIS) in patients with gemcitabine-refractory pancreatic cancer.

Subcategory:
Multidisciplinary Treatment

Category:
Cancers of the Pancreas Small Bowel and Hepatobiliary Tract

Meeting:
2013 Gastrointestinal Cancers Symposium

Session Type and Session Title:
General Poster Session B: Cancers of the Pancreas, Small Bowel, and Hepatobiliary Tract

Abstract Number:
263

Citation:
J Clin Oncol 31, 2013 (suppl 4; abstr 263)

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Abstracts that were granted an exception in accordance with ASCO's Conflict of Interest Policy are designated with a caret symbol (^).

Abstract Disclosures

Abstract:

Background: Gemcitabine (Gem) monotherapy or Gem-based combination therapy is a standard first-line therapy for advanced pancreatic cancer (PC). There is no consensus on second-line therapy in patients (pts) with disease progression (PD) after Gem-based therapy. S-1, an oral fluoropyrimidine derivative, is commonly used for the second-line treatment of PC in Japan. Shitara et al previously reported that IRIS regimen showed that 44% of response rate (RR), 4.9 mo of median progression free survival (PFS), and 11.3 mo of median overall survival (OS), respectively. Therefore a randomized phase II trial was conducted to evaluate the efficacy and safety of IRIS compared with S-1 alone in the second-line setting. **Methods:** The inclusion criteria were as follows: (1) histologically or cytologically proven pancreatic adenocarcinoma or adenosquamous carcinoma; (2) confirmed PD after Gem treatment; (3) ECOG PS, 0-1; (4) measurable metastatic lesion based on RECIST criteria; (5) age ≥ 20 years; (6) total bilirubin < 2.0 mg/dL. Patients were randomized to receive either IRIS (CPT-11 100 mg/m^2, iv, d1,15 plus S-1 80/100/120 mg/day based on BSA, po, d1-14, q4w; Arm A) or S-1 (80/100/120 mg/day based on BSA, po, d1-28, q6w; Arm B). The primary endpoint was to compare PFS in Arm A and Arm B. **Results:** Of a total of 137 pts enrolled between Nov 2008 and Mar 2011, 127 were eligible

(60 randomized to Arm A and 67 to B). Median PFS in Arm A and B was 107 and 58 days, respectively (HR= 0.767; 95% CI, 0.527-1.114; p=0.1750). Median OS in Arm A and B was 208 and 176 days, respectively (HR=0.749; 95% CI, 0.512-1.093; p=0.1338). RR was 18.3% in Arm A (11/60; 95% CI, 9.5-30.4) and 6.0% in Arm B (4/67; 95% CI, 1.7-14.6) (p=0.0311). The incidences of grade 3/4 toxicities were as follows: neutropenia (15.6% and 4.3%), anorexia (23.4% and 17.3%), nausea (6.3% and 2.9%), and diarrhea (3.1% and 2.9%) in Arm A and B, respectively. Both regimens were tolerable. **Conclusions:** Although IRIS showed no significant improvement in PFS or OS compared with S-1 alone in this study, it showed significant advantage in RR, and favorable HR in both of PFS and OS. IRIS might have potential power to treat second-line PC patients. Further study is warranted. Clinical trial information: JapicCTI-080657.

Abstracts by Nobumasa Mizuno:

Is serum HER2-ECD testing significant for resectable gastric cancer?
Meeting: 2015 Gastrointestinal Cancers Symposium | **Abstract No:** 48 | **First Author:** Tsutomu Tanaka
Category: Cancers of the Esophagus and Stomach - Prevention, Diagnosis, and Screening

Randomized phase III study of etoposide plus cisplatin versus irinotecan plus cisplatin in advanced neuroendocrine carcinoma of the digestive system: A Japan Clinical Oncology Group study (JCOG1213).
Meeting: 2015 ASCO Annual Meeting | **Abstract No:** TPS4143 | **First Author:** Chigusa Morizane
Category: Gastrointestinal (Noncolorectal) Cancer - Neuroendocrine/Carcinoid

Randomized phase III study of gemcitabine plus S-1 combination therapy versus gemcitabine plus cisplatin combination therapy in advanced biliary tract cancer: A Japan Clinical Oncology Group study (JCOG1113).
Meeting: 2014 ASCO Annual Meeting | **Abstract No:** TPS4149 | **First Author:** Chigusa Morizane
Category: Gastrointestinal (Noncolorectal) Cancer - Hepatobiliary Cancer

More

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 263266-411935	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2016/047727	International filing date *(day/month/year)* 19 August 2016 (19.08.2016)	Priority date *(day/month/year)* 21 August 2015 (21.08.2015)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant IPSEN BIOPHARM LTD.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☐	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 27 February 2018 (27.02.2018)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Agnès Wittmann-Regis e-mail: pct.team6@wipo.int

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PATENT COOPERATION TREATY

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INTERNATIONAL SEARCHING AUTHORITY

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
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Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2016/047727	19.08.2016	21.08.2015

International Patent Classification (IPC) or both national classification and IPC
INV. A61K31/436 A61K9/127 A61K31/282 A61K31/4745 A61K31/475 A61K31/513 A61K31/519 A61P35/04

Applicant
MERRIMACK PHARMACEUTICALS, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Engl, Brigitte Telephone No. +49 89 2399-0

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

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4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-15
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-15
Industrial applicability (IA)	Yes:	Claims	1-15
	No:	Claims	

2. Citations and explanations

see separate sheet

Box V:

1 Prior Art

The following prior art documents are cited from the International Search Report:

D1 CHANG T C ET AL: "Phase I study of nanoliposomal irinotecan (PEP02) in advanced solid tumor patients", CANCER CHEMOTHERAPY AND PHARMACOLOGY, SPRINGER VERLAG, BERLIN, vol. 75, no. 3, 11 January 2015 (2015-01-11), pages 579-586, XP035456963, ISSN: 0344-5704, DOI: 10.1007/S00280-014-2671-X

D2 L. Chen, H. Shiah, T. Chao, R. K. Hsieh, G. Chen, J. Chang, G. Yeh: "Phase I study of liposome irinotecan (PEP02) in combination with weekly infusion of 5-FU/LV in advanced solid tumors", Journal of Clinical Oncology, vol. 28, no. 15_Suppl., E13024, 2010, XP002763720, DOI: 10.1200/jco.2010.28.15_suppl.e13024 Retrieved from the Internet: URL: http://ascopubs.org/doi/abs/10.1200/jco.2010.28.15_suppl.e13024 [retrieved on 2016-11-02]

D3 KO A H ET AL: "A multinational phase 2 study of nanoliposomal irinotecan sucrosofate (PEP02, MM-398) for patients with gemcitabine-refractory metastatic pancreatic cancer", BRITISH JOURNAL OF CANCER 20 AUG 2013, vol. 109, no. 4, 20 August 2013 (2013-08-20), pages 920-925, XP002763721, ISSN: 1532-1827

D4 PETER J HOSEIN ET AL: "A retrospective study of neoadjuvant FOLFIRINOX in unresectable or borderline-resectable locally advanced pancreatic adenocarcinoma", BMC CANCER, BIOMED CENTRAL, LONDON, GB, vol. 12, no. 1, 29 May 2012 (2012-05-29), page 199, XP021126474, ISSN: 1471-2407, DOI: 10.1186/1471-2407-12-199

D1 describes nanoliposomal irinotecan and its maximum tolerated dose (MTD) of 120 mg/m^2 as monotherapy at a 3-week interval.

D2 describes a phase I study of liposome irinotecan as 90 mins i.v. infusion on D1 in combination with 24-hr infusion of 5-fluorouracil (5-FU) (2,000 mg/m^2)/ leucovorin (LV) (200 mg/m^2) on D1 and D8 every 3 weeks, wherein cohorts of 3-6 pts were treated at 60, 80, 100, and 120 mg/m^2.

D3 describes a phase 2 study of nanoliposomal irinotecan (PEP02, MM-398) for patients with gemcitabine-refractory metastatic pancreatic cancer and reports a study testing oxaliplatin, 5-FU and folinic acid (= leucovorin) (OFF) versus 5-FU/folinic acid alone for the second-line treatment of advanced pancreatic cancer. The study showed that patients receiving the oxaliplatin-combination demonstrated significantly improved outcomes in terms of both progression-free survival and overall survival.

D4 describes a study of 5-fluorouracil, leucovorin, irinotecan and oxaliplatin (FOLFIRINOX) as neoadjuvant therapy in patients with unresectable locally advanced pancreatic adenocarcinoma.

2 Novelty

The present claimed combination of liposomal irinotecan, oxaliplatin, 5-fluorouracil and leucovorin for use in treating metastatic adenocarcinoma of the pancreas in patients not having previously received chemotherapy differs from that described in **D4** in that presently liposomal irinotecan, rather than irinotecan, is used. The claimed subject-matter is therefore considered novel (Article 33(2) PCT).

3 Inventive Step

3.1 The underlying problem was the provision of a combination for treating metastatic adenocarcinoma of the pancreas in patients not having previously received chemotherapy for treating the said condition.

3.2 The claimed solution is the combination of liposomal irinotecan, oxaliplatin, 5-fluorouracil and leucovorin for use in treating the said condition according to the treatment schedule specified in the claims.

3.3 The claimed solution is considered to be *prima facie* obvious in the light of the prior art since both liposomal irinotecan and combination thereof with 5-FU and leucovorin, on the one hand, as well as the benefit of adding oxaliplatin to a combination treatment has been known in the art. It is therefore held that a skilled practitioner would have arrived at the present combination and use by applying nothing more than routine experimentation. Therefore, inventive step (Article 33(3) PCT) cannot be acknowledged in the absence of an unexpected effect shown for the claimed matter.

4 Industrial Applicability

The claimed subject-matter is industrially applicable (Article 33(4) PCT) in the medical and pharmaceutical field. The claims might be found objectionable because they are directed to methods of treatment by therapy.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
239669-401117		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2016/047727	19 August 2016 (19-08-2016)	21 August 2015 (21-08-2015)
Applicant		
MERRIMACK PHARMACEUTICALS, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language,** the international search was carried out on the basis of:
      [X] the international application in the language in which it was filed
      [ ] a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
   b. [ ] This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. [ ] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. [ ] **Certain claims were found unsearchable** (See Box No. II)

3. [ ] **Unity of invention is lacking** (see Box No III)

4. With regard to the **title,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the **drawings,**
   a. the figure of the **drawings** to be published with the abstract is Figure No. _____
      [ ] as suggested by the applicant
      [ ] as selected by this Authority, because the applicant failed to suggest a figure
      [ ] as selected by this Authority, because this figure better characterizes the invention
   b. [X] none of the figures is to be published with the abstract

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/047727

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/436 A61K9/127 A61K31/282 A61K31/4745 A61K31/475
A61K31/513 A61K31/519 A61P35/04
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, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHANG T C ET AL: "Phase I study of nanoliposomal irinotecan (PEP02) in advanced solid tumor patients", CANCER CHEMOTHERAPY AND PHARMACOLOGY, SPRINGER VERLAG, BERLIN, vol. 75, no. 3, 11 January 2015 (2015-01-11), pages 579-586, XP035456963, ISSN: 0344-5704, DOI: 10.1007/S00280-014-2671-X [retrieved on 2015-01-11] the whole document ----- -/--	1-15

[X] 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
2 November 2016	16/11/2016

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	Engl, Brigitte

1

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.
Y	L. Chen, H. Shiah, T. Chao, R. K. Hsieh, G. Chen, J. Chang, G. Yeh: "Phase I study of liposome irinotecan (PEP02) in combination with weekly infusion of 5-FU/LV in advanced solid tumors", Journal of Clinical Oncology, vol. 28, no. 15_Suppl., E13024, 2010, XP002763720, DOI: 10.1200/jco.2010.28.15_suppl.e13024 Retrieved from the Internet: URL:http://ascopubs.org/doi/abs/10.1200/jco.2010.28.15_suppl.e13024 [retrieved on 2016-11-02] abstract -----	1-15
Y	KO A H ET AL: "A multinational phase 2 study of nanoliposomal irinotecan sucrosofate (PEP02, MM-398) for patients with gemcitabine-refractory metastatic pancreatic cancer", BRITISH JOURNAL OF CANCER 20 AUG 2013, vol. 109, no. 4, 20 August 2013 (2013-08-20), pages 920-925, XP002763721, ISSN: 1532-1827 page 920, left-hand column, line 1 - page 921, left-hand column, line 43 page 923, right-hand column, line 12 - page 924, left-hand column, line 67 -----	1-15
Y	PETER J HOSEIN ET AL: "A retrospective study of neoadjuvant FOLFIRINOX in unresectable or borderline-resectable locally advanced pancreatic adenocarcinoma", BMC CANCER, BIOMED CENTRAL, LONDON, GB, vol. 12, no. 1, 29 May 2012 (2012-05-29), page 199, XP021126474, ISSN: 1471-2407, DOI: 10.1186/1471-2407-12-199 the whole document -----	1-15

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2016/047727	19.08.2016	21.08.2015

International Patent Classification (IPC) or both national classification and IPC
INV. A61K31/436 A61K9/127 A61K31/282 A61K31/4745 A61K31/475 A61K31/513 A61K31/519 A61P35/04

Applicant
MERRIMACK PHARMACEUTICALS, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Engl, Brigitte Telephone No. +49 89 2399-0

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-15
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-15
Industrial applicability (IA)	Yes:	Claims	1-15
	No:	Claims	

2. Citations and explanations

see separate sheet

Box V:

1 Prior Art

The following prior art documents are cited from the International Search Report:

D1 CHANG T C ET AL: "Phase I study of nanoliposomal irinotecan (PEP02) in advanced solid tumor patients", CANCER CHEMOTHERAPY AND PHARMACOLOGY, SPRINGER VERLAG, BERLIN, vol. 75, no. 3, 11 January 2015 (2015-01-11), pages 579-586, XP035456963, ISSN: 0344-5704, DOI: 10.1007/S00280-014-2671-X

D2 L. Chen, H. Shiah, T. Chao, R. K. Hsieh, G. Chen, J. Chang, G. Yeh: "Phase I study of liposome irinotecan (PEP02) in combination with weekly infusion of 5-FU/LV in advanced solid tumors", Journal of Clinical Oncology, vol. 28, no. 15_Suppl., E13024, 2010, XP002763720, DOI: 10.1200/jco.2010.28.15_suppl.e13024 Retrieved from the Internet: URL: http://ascopubs.org/doi/abs/10.1200/jco.2010.28.15_suppl.e13024 [retrieved on 2016-11-02]

D3 KO A H ET AL: "A multinational phase 2 study of nanoliposomal irinotecan sucrosofate (PEP02, MM-398) for patients with gemcitabine-refractory metastatic pancreatic cancer", BRITISH JOURNAL OF CANCER 20 AUG 2013, vol. 109, no. 4, 20 August 2013 (2013-08-20), pages 920-925, XP002763721, ISSN: 1532-1827

D4 PETER J HOSEIN ET AL: "A retrospective study of neoadjuvant FOLFIRINOX in unresectable or borderline-resectable locally advanced pancreatic adenocarcinoma", BMC CANCER, BIOMED CENTRAL, LONDON, GB, vol. 12, no. 1, 29 May 2012 (2012-05-29), page 199, XP021126474, ISSN: 1471-2407, DOI: 10.1186/1471-2407-12-199

D1 describes nanoliposomal irinotecan and its maximum tolerated dose (MTD) of 120 mg/m^2 as monotherapy at a 3-week interval.

D2 describes a phase I study of liposome irinotecan as 90 mins i.v. infusion on D1 in combination with 24-hr infusion of 5-fluorouracil (5-FU) (2,000 mg/m^2)/ leucovorin (LV) (200 mg/m^2) on D1 and D8 every 3 weeks, wherein cohorts of 3-6 pts were treated at 60, 80, 100, and 120 mg/m^2.

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D4 describes a study of 5-fluorouracil, leucovorin, irinotecan and oxaliplatin (FOLFIRINOX) as neoadjuvant therapy in patients with unresectable locally advanced pancreatic adenocarcinoma.

2 Novelty

The present claimed combination of liposomal irinotecan, oxaliplatin, 5-fluorouracil and leucovorin for use in treating metastatic adenocarcinoma of the pancreas in patients not having previously received chemotherapy differs from that described in **D4** in that presently liposomal irinotecan, rather than irinotecan, is used. The claimed subject-matter is therefore considered novel (Article 33(2) PCT).

3 Inventive Step

3.1 The underlying problem was the provision of a combination for treating metastatic adenocarcinoma of the pancreas in patients not having previously received chemotherapy for treating the said condition.

3.2 The claimed solution is the combination of liposomal irinotecan, oxaliplatin, 5-fluorouracil and leucovorin for use in treating the said condition according to the treatment schedule specified in the claims.

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4 Industrial Applicability

The claimed subject-matter is industrially applicable (Article 33(4) PCT) in the medical and pharmaceutical field. The claims might be found objectionable because they are directed to methods of treatment by therapy.

NAPOLI 1: Randomized Phase 3 Study of MM-398 (nal-IRI), With or Without 5-Fluorouracil and Leucovorin, Versus 5-Fluorouracil and Leucovorin, in Metastatic Pancreatic Cancer Progressed on or following Gemcitabine-Based Therapy

D. Von Hoff,[1] C.-P. Li,[2] A. Wang-Gillam,[3] G. Bodoky,[4] A. Dean,[5] G. Jameson,[1] T. Macarulla,[6] K-H. Lee,[7] D. Cunningham,[8] J-F. Blanc,[9] R. Hubner,[10] C-F. Chiu,[11] G. Schwartsmann,[12] J. Siveke,[13] F. Braiteh,[14] V. Moyo,[15] B. Belanger,[15] N. Dhindsa,[15] E. Bayever,[15] L-T. Chen[16]

[1]TGen, Scottsdale Healthcare, Scottsdale, AZ, USA; [2]Taipei Veterans General Hospital and National Yang-Ming University, Taipei, Taiwan; [3]Washington University, St Louis, MO, USA; [4]Szent László Teaching Hospital, Budapest, Hungary; [5]St John of God Hospital, Subiaco, Western Australia, Australia; [6]Vall d'Hebron University Hospital, Barcelona, Spain; [7]Seoul National University Hospital, Seoul, South Korea; [8]The Royal Marsden Hospital, London, UK; [9]Hôpital Saint-André, Bordeaux, France; [10]The Christie NHS Foundation Trust, Manchester, UK; [11]China Medical University Hospital, Taichung, Taiwan; [12]Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil; [13]Klinikum rechts der Isar der TU München, Munich, Germany; [14]Comprehensive Cancer Centers of Nevada, Las Vegas, NV, USA; [15]Merrimack Pharmaceuticals Inc., Cambridge, MA, USA; [16]National Cheng Kung University Hospital, Tainan City, Taiwan

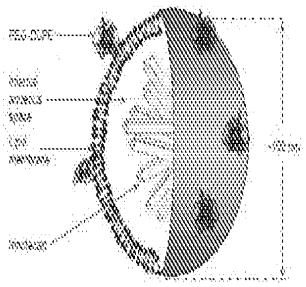
BACKGROUND

Pancreatic Cancer

Pancreatic cancer is the fourth-leading cause of cancer death in the EU and United States.[1,2] There is no standard of care for patients with metastatic pancreatic cancer who have been previously treated with a gemcitabine-based regimen.

MM-398: Nanoliposomal Irinotecan (nal-IRI)*

- MM-398 (120 mg/m²) clinical PK showed extended circulation
  - ~ 70x higher AUC of total irinotecan in blood vs conventional irinotecan (300 mg/m²)[3]
- MM-398 achieved 5x higher levels of SN-38 (active metabolite) in tumor compared to blood at 72 hours[4]
- Median OS of 5.2 months in Phase 2 study of gemcitabine-refractory metastatic pancreatic cancer[5]



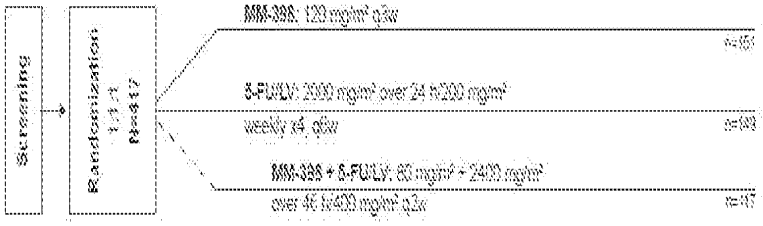
*Also known as PEP02, Pharmaessentia Inc., Taiwan

STUDY OBJECTIVES & STUDY DESIGN

- Primary: Overall survival (OS)
- Secondary: Include progression-free survival (PFS), objective response rate (ORR), tumor marker response (CA19-9), and safety

Study Amendment

Study was amended to add the MM-398 + 5-FU/LV arm once safety data on the combination became available; 63 patients had been enrolled in the original 2-arm study at the time of amendment.



METHODOLOGY

- Open label, randomized, stratified by albumin (<4.0 g/dL vs ≥4.0 g/dL), KPS (70 and 80 vs ≥90), and ethnicity (Caucasian vs East Asian vs others)
- Each treatment arm was compared to its corresponding 5-FU/LV control for OS by unstratified log-rank test; family-wise type I error rate was controlled at the 2-sided 0.05 level using the Bonferroni-Holm method
- Primary analysis planned when at least 305 death events occurred to have 85% power to detect HR=0.67 in the MM-398 arm and 38% power to detect HR=0.5 in the MM-398 + 5-FU/LV arm

Key Inclusion Criteria

- Adenocarcinoma of the exocrine pancreas
- Metastatic disease, measurable or non-measurable
- Progressed after prior gemcitabine or gemcitabine-containing therapy
- KPS ≥70
- Adequate bone marrow, hepatic (bilirubin within normal range for the institution, and albumin ≥3 g/dL), and renal function

PATIENT CHARACTERISTICS & TREATMENT EXPOSURE

Patient Characteristics at Baseline		MM-398 + 5-FU/LV (n=117)	5-FU/LV Post Liposomal (n=117)	MM-398 (n=121)	5-FU/LV (n=122)
Age	Median years (min, max)	62 (41, 81)	62 (34, 80)	65 (31, 87)	63 (30, 89)
Sex	Male, %	59	56	58	54
KPS	90-100, %	56	55	55	56
	70-80, %	44	44	44	44
Ethnicity	Caucasian, %	64	65	60	66
	East Asian, %	29	30	36	34
	Other, %	7	7	5	2
Pancreatic primary location	Head, %	64	63	65	64
	Other, %	36	37	35	36
CA19-9*	>37 U/mL, %	81	88	82	77

*CA19-9 at baseline was unknown in 3% of patients

	MM-398 + 5-FU/LV (n=117)	5-FU/LV Post Liposomal (n=117)	MM-398 (n=121)	5-FU/LV (n=122)
Prior lines of treatment, %				
1	42	45	46	47
>1	57	55	54	53
Neoadj only	13	13	11	13
Neoadj + 1	11	12	12	11
Neoadj + >1	7	3	6	3
Gemcitabine alone	45	46	44	44
Gemcitabine combo	55	54	56	56
Post study anticancer therapy, %				
Any therapy, %	31	38	36	37
Gemcitabine-based	9	10	11	11
5-FU-based	18	25	20	20
Irinotecan-based	7	8	5	7
Platinum-based	15	13	17	15
Investigational	3	3	3	3
Other	10	8	15	7

Enrollment of 417 patients at 105 sites (Jan 2012 to Sep 2013)

EU (Czech Rep., France, Germany, Hungary, Italy, Spain, UK): 37%, Asia (South Korea, Taiwan): 32%, N. America (Canada, USA): 17%, Australia: 10%, S. America (Argentina, Brazil): 4%.

- Treatment duration (weeks), median (min, max): MM-398 + 5-FU/LV: 7.1 (0.4, 63), MM-398: 6.0 (0.1, 40), 5-FU/LV: 3.3 (0.3, 67)
- Relative dose intensity (%), median (min, max): MM-398 + 5-FU: 88 (29, 107) + 91 (32, 115), MM-398: 95 (59, 105), 5-FU: 100 (35, 108)

EFFICACY

Figure 1: OS (MM-398 + 5-FU/LV)

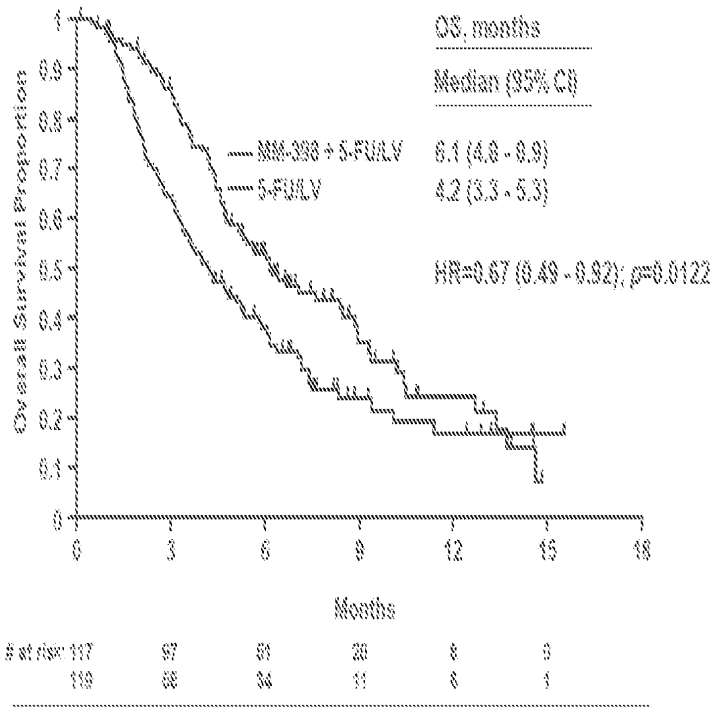


Figure 2: PFS (MM-398 + 5-FU/LV)

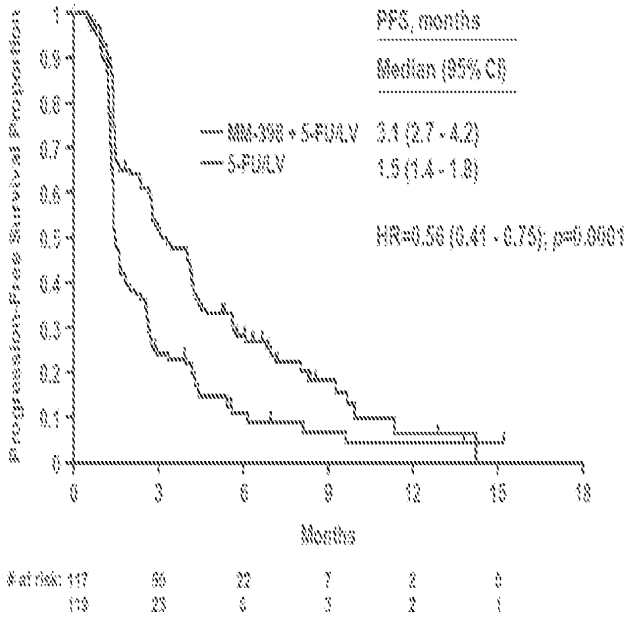
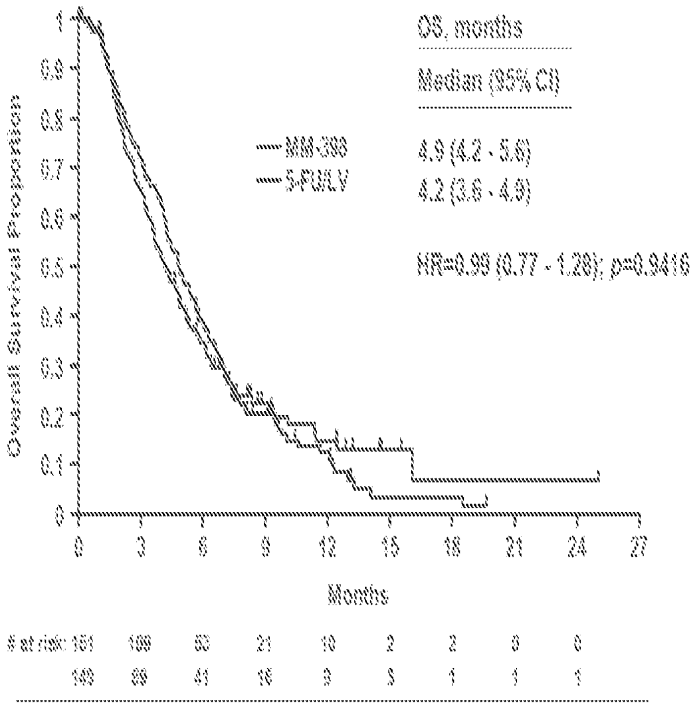


Figure 3: OS (MM-398 Monotherapy)



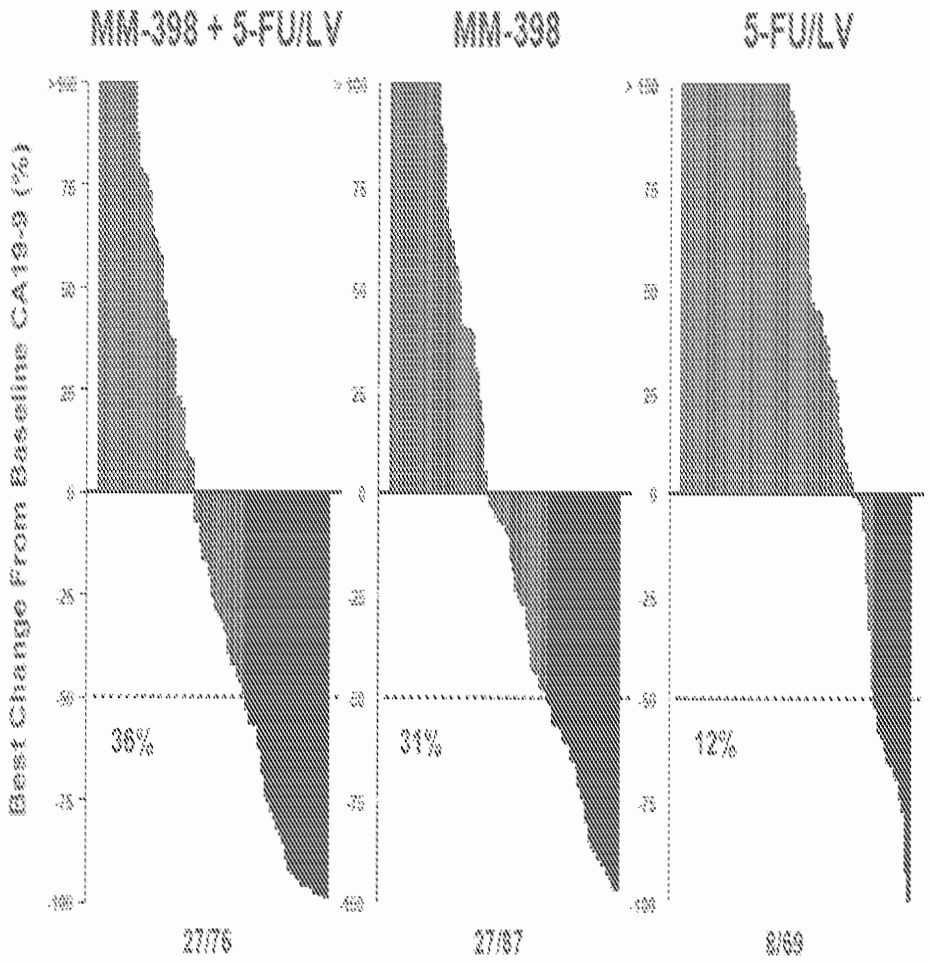
■ Data cut-off date was February 14, 2014, on which the sponsor received notification of the 305th event. Final analysis included 313 death events. Data were censored if patients were alive or lost to follow-up at the time of analysis

Figure 4: Tumor Response and Control

	MM-398 + 5-FU/LV (n=117)	5-FU/LV Post Amendment (n=119)
Overall response rate, %[3]	16	1
(95% CI)	(9.6-22.9)	(0.0-2.5)
p-value	<0.001	
PFS rate at 12 weeks, %	57	26
(95% CI)	(47-66)	(18-35)
	MM-398 (n=151)	**5-FU/LV All (n=149)**
Overall response rate, %[3]	6	1
(95% CI)	(2.2-9.7)	(0-2.0)
p-value	0.019	
PFS rate at 12 weeks, %	47	28
(95% CI)	(38-55)	(21-36)

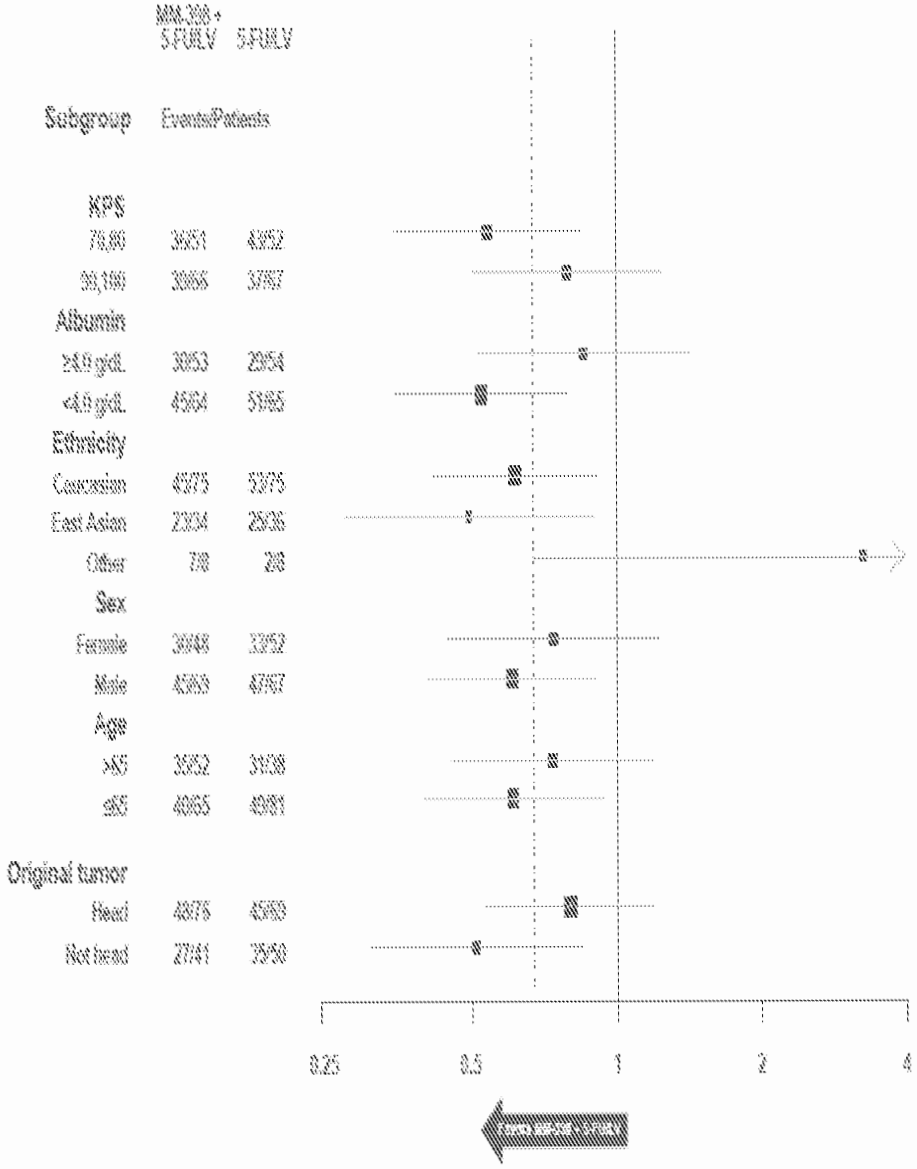
[3] Per RECIST version 1.1.

Figure 5: Tumor Marker Response (CA19-9)*



*Response defined as ≥50% reduction in baseline CA19-9 levels, in patients with baseline levels >30 U/mL and at least one post-baseline CA19-9 measurement.

Figure 6: Forest Plot—MM-398 + 5-FU/LV



SUMMARY OF EFFICACY RESULTS

- Fig. 1: OS improved significantly on MM-398 80 mg/m^2 q2w + 5-FU/LV
- Fig. 2: PFS improved significantly on MM-398 80 mg/m^2 q2w + 5-FU/LV
- Fig. 3: OS and PFS (not shown) not statistically improved on MM-398 120 mg/m^2 q3w

- Fig. 4: ORR improved significantly on MM-398 + 5-FU/LV
- Fig. 5: Highest CA19-9 response was seen with MM-398 + 5-FU/LV; monotherapy (MM-398) also showed activity
- Fig. 6: A consistent treatment effect was observed across all sub-groups

SAFETY: HEMATOLOGIC

Safety Variable	MM-398 + 5-FU/LV (n=117)	MM-398 (n=147)	5-FU/LV Arm (n=134)
Grade 3/4 Hematologic AEs Based on Laboratory Values, %[a,b]			
Neutrophil count decreased	20	16	2
Hemoglobin decreased	6	7	5
Platelet count decreased	2	1	0
Febrile Neutropenia, %	2	4	0
Patients Who Received Growth Factors, %	17	12	1

[a]Includes only patients who had at least one post-baseline assessment.
[b]Per CTCAE version 4.

- Adverse events seen were consistent with the known safety profile of the constituent drugs

SAFETY: NON-HEMATOLOGIC

Safety Variable	MM-398 + 5-FU/LV (n=117)	MM-398 (n=147)	5-FU/LV Alt (n=134)
Grade ≥3 Non-hematologic AEs in >5% of Patients, %*			
Fatigue	14	6	4
Diarrhea	13	21	5
Vomiting	11	14	3
Nausea	8	5	3
Asthenia	8	7	7
Abdominal Pain	7	8	6
Decreased Appetite	4	3	2
Hypokalemia	3	12	2
Hypernatremia	3	6	2

*Per CTCAE version 4.

CONCLUSIONS

- MM-398, at 80 mg/m^2 q2w + 5-FU/LV, superior for OS, PFS, ORR, and CA19-9 responses
- Most frequent Grade 3+ AEs for the combination include neutropenia, fatigue, and GI effects (diarrhea and vomiting)
- MM-398, as a single-agent at 120 mg/m^2 q3w, showed activity (tumor response and reduction in CA19-9 levels) but no statistically significant improvement in OS or PFS
- MM-398 + 5-FU/LV demonstrated promising clinical activity in patients with metastatic pancreatic cancer after gemcitabine-based treatment

References: 1. Ferlaya J et al. *Eur J Cancer*. 2013;49(6):1374-1403. 2. American Cancer Society. Cancer Facts and Figures 2014. Atlanta: American Cancer Society; 2014. 3. Roy AC et al. *Ann Oncol*. 2013;24(6):1567-1573. 4. Ramanathan RK et al. Proc.105th AACR; 2014. CT224. 5. Ko AH et al. *Br J Cancer*. 2013;109(4):920-925.

Acknowledgments: We would like to thank all patients, their families, caregivers, investigators, and research staff for their participation.

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EXAMINER	
SHOMER, ISAAC	

ART UNIT	PAPER NUMBER
1612	

MAIL DATE	DELIVERY MODE
08/17/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	11/121,294	HONG ET AL.	
	Examiner	Art Unit	
	ISAAC SHOMER	1612	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *26 May 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *13-22,24-58,61-66 and 68-164* is/are pending in the application.

4a) Of the above claim(s) *See Continuation Sheet* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *13-22,24-28,30-32,34-36,39-50,52-57,94-116,119,156-158,161,162 and 164* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *See Continuation Sheet*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Continuation of Disposition of Claims: Claims withdrawn from consideration are 29,33,37,38,51,58,61-66,68-93,117,118,120-155,159-160 and 163.

Continuation of Attachment(s) 3). Information Disclosure Statement(s) (PTO/SB/08), Paper No(s)/Mail Date :30 September 2005, 29 December 2008, 11 May 2009.

DETAILED ACTION

Election/Restrictions - Groups

Applicant's election of Group I, claims 13-22, 24-57 94-119, 156-158 161-162 and 164 in the reply filed on 26 May 2009 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Claims 58, 61-66, 68-93, 120-155, 159-160 and 163 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 26 May 2009. Claims 160 and 163 are withdrawn as depending upon non-elected claim 66.

Election/Restrictions

Applicant's election with traverse of the following species in the reply filed on 26 May 2009 is acknowledged:

Sucrose octasulfate is elected with respect to the polyanion.

A gradient of substituted ammonium ions comprising at least one C-N bond is elected as to the transmembrane gradient.

Sucrose is elected as to the polyol moiety.

Sulfuric acid ester is elected as to the strongly anionic functional group.

Irinotecan and camptothecin prodrug are elected as to the therapeutic entity.

Triethylammonium is elected as to the substituted ammonium compound.

Mouse is elected as to the mammal as to claims 55 and 57.

Polyethylene glycol is elected as to the hydrophilic polymer, and a PEG-derivatized phospholipid is elected as to the hydrophilic polymer derivatized lipid.

Rat is elected as to the mammal as to claims 97-98.

An antibody sequences is elected as to the targeting moiety.

ErbB-2 is elected as the tyrosine kinase growth factor receptor.

VEGF receptor is elected as to the angiogenic factor receptor.

The examiner withdraws the species elections on “mammal” (wherein applicant elected “mouse” as to claims 44 and 57 and “rat” as to claims 97-98). The examiner further expands the species election on the active agent to include doxorubicin. The examiner expands the species election regarding the transmembrane gradient to include all disclosed species of claim 14.

The traversal is on the ground(s) that there is no search burden. Specifically, applicant believes that all types of transmembrane gradients, such as ammonium ion gradients and pH gradients would be found in the same source, and all of the species of polyanionized sugars would not require different fields of search. This is not found persuasive because different transmembrane gradients and different polyanions have different physiological effects. For example, the elected polyanion, sucrose octasulfate, has physiological properties that protect the stomach from ulcers, which are not present

in the case of other polyanions. Different active agents have distinct physiological uses, and therefore require different fields of search.

The requirement is still deemed proper and is therefore made FINAL.

Claims 29, 33, and 37-38 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected species, (specifically that of irinotecan), there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 26 May 2009.

Claim 51 is withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected specie, specifically that of sucrose as the polyol moiety and sulfate (sulfuric acid esters) as the strongly anionic functional group, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 26 May 2009.

Claims 117-118 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected specie, specifically that of a tyrosine kinase receptor, wherein said receptor is elected to be ErbB-2, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 26 May 2009.

Claims 13-22, 24-28, 30-32, 34-36, 39-50, 52-57, 94-116, 119, 156-158 161-162 and 164 are under substantive examination.

Claim Objections

Applicant is advised that should claim 19 be found allowable, claim 20 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k).

Claim Rejections - 35 USC § 112 2nd Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 13-22, 24-28, 30-32, 34-36, 39-50, 51-57, 94-116, 119, 156-158 and 164 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention:

Claims 21, 24, 40, 54 and 164 recite the limitation “at least about." The term “at least” delineates only numerical values more than the recited value where the term “about” may be less than or more than the recited value. Because of the conflict of terms, it is unclear which term is limiting. See also MPEP 2173.05(b) (citing Amgen v. Chugai, 18 USPQ2d 1016 (Fed. Cir. 1991), in which the phrase “at least about” was held indefinite).

Where applicant acts as his or her own lexicographer to specifically define a term of a claim contrary to its ordinary meaning, the written description must clearly redefine the claim term and set forth the uncommon definition so as to put one reasonably skilled in the art on notice that the applicant intended to so redefine that claim term. *Process Control Corp. v. HydReclaim Corp.*, 190 F.3d 1350, 1357, 52 USPQ2d 1029, 1033 (Fed. Cir. 1999).

The term “wherein said liposome comprises a transmembrane gradient” in claim 13 is used by the claim to mean “wherein a transmembrane gradient exists between the liposome and the medium”, while the accepted meaning is “wherein a transmembrane gradient exists only in the confines of the liposome.” The term is indefinite because the specification does not clearly redefine the term. The term "gradient" is known in the art to refer to one entity changing as a function of some effect. For example, a gradient used for an HPLC mobile phase would refer to the mobile phase composition as a function of time. The examiner understands the claimed gradient to read on the concentration of a solute (e.g. substituted ammonium ions, hydrogen ions etc.) changing with respect to location (e.g. inside or outside the liposome). The claims as recited do not clarify either of the variables being altered.

The phase “wherein said liposome composition has an anti-neoplastic activity at least [a] four-fold higher than that of the anti-neoplastic activity of the entity in a free non-liposomal form,” is indefinite with regard to claims 43-57. This is because claims 43-57 define said entity to be a pro-drug. It is the understanding of the examiner that the term pro-drug reads on an entity which has no therapeutic activity itself, but is

metabolized into a different entity with therapeutic activity. Therefore, it is not clear whether the anti-neoplastic activity refers to that of the pro-drug or that of the active metabolite. If applicant defines the term pro-drug to read on both the administered entity and the active metabolite, this definition is contrary to the accepted meaning of the term “pro-drug.” The indefinite term "anti-neoplastic therapeutic entity," is examined under the art as if it reads on both the pro-drug and its active metabolite.

The phrase “gram-equivalent,” of claims 21-22 is indefinite, as it is not defined what a "gram-equivalent" is or how it differs from a gram. For the purpose of examination, a gram-equivalent will be defined as a gram.

Claim Rejections - 35 USC § 112 1st Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 13, 15, 24, 28, 30-32, 40, 43-44, 46-50, and 52-58 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The description requirement of the patent statute requires a description of an invention, not an indication of a result that one might achieve if one made that invention.

See, e.g., In re Wilder, 22 USPQ 369, 372-3 (Fed. Cir. 1984). (Holding that a claim was not adequately described because the specification did 'little more than outline goals appellants hope the claimed invention achieves and the problems the invention will hopefully ameliorate.')

Mere indistinct terms (such as "prodrug" "derivative" and "analog" used in claims 29-33 and 43-44), however, may not suffice to meet the written description requirement. This is particularly true when a compound is claimed in purely functional terms. See Univ. of Rochester v. G.D. Searle, 69 USPQ2d 1886 (CAFC 2004) at 1892, stating:

> The appearance of mere indistinct words in a specification or a claim, even an original claim, does not necessarily satisfy that requirement. A description of an anti-inflammatory steroid, i.e., a steroid (a generic structural term) described even in terms of its functioning of lessening inflammation of tissues fails to distinguish any steroid from others having the same activity or function. A description of what a material does, rather than of what it is, usually does not suffice…. The disclosure must allow one skilled in the art to visualize or recognize the identity of the subject matter purportedly described. (Emphasis added).

Conversely, a description of a chemical genus will usually comprise a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus. See Univ. of Calf. V. Eli Lilly, 43 USPQ 2d 1398, 1406 (Fed. Cir. 1997). This is analogous to enablement of a genus under Section 112, ¶ 1, by showing the enablement of a representative number of species within the genus.

A chemical genus can be adequately described if the disclosure presents a sufficient number of representative species that encompass the genus. *If the genus has substantial variance, the disclosure must describe a sufficient number of species to reflect the variation within that genus*. See MPEP 2163. The MPEP lists factors that can be used to determine if sufficient evidence of possession has been furnished in the disclosure of the Application. These include the level of skill and knowledge in the art,

partial structure, physical and/or chemical properties, functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the method of making the claimed invention. Disclosure of any *combination of such identifying characteristics that distinguish the claimed invention from other materials* and would lead one of skill in the art to the conclusion that the applicant was in possession of the claimed species is sufficient. MPEP 2163.

Here, the specification does not provide a reasonably representative disclosure of useful drugs generally, a potentially huge genus inclusive of many different compounds having widely divergent structures and functions. Specifically, the specification discloses only a limited number of species at paragraphs 0087 through 0092, and these are not viewed as being reasonably representative of the genus in its claimed scope because no readily apparent combination of identifying characteristics is provided, other than the disclosure of those specific species as examples of the claimed genus.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

For the purpose of examination under the art, the indefinite term "gram-equivalent/L" of claims 21-22 will be examined as grams per liter, or mg/mL.

Claims 13-16, 21-22, 24-28, 30-32, 34-36, 39-42, 94-95, 97-98 and 100-101 are rejected under 35 U.S.C. 102(b) as being anticipated by Kirpotin (US Patent 6,110,491).

Kirpotin discloses that liposomes loaded with the presence of polymeric anionic compounds such as polysaccharide sulfate (chondroitin sulfate, heparin etc.) results in an increase of the concentration of encapsulated compound by several fold. Kirpotin teaches the presence of a gradient of charged polymer as of Kirpotin, column 9 lines 12-16. The examiner points to Kirpotin Example 1 (column 12 lines 5-36), which shows a doxorubicin ratio of 8 nmol/micromole for liposomes loaded without a polymeric sugar and in Example 3, (column 12 line 62 to column 13 lines 1-6, which also refers back to Example 1) a loading ratio of 26 nmol/micromole with the use of chondroitin sulfate. Said liposomes comprise 5 mg/mL (which is equivalent to grams per liter) of chondroitin sulfate in the inner buffer (i.e. liposome interior), as of Kirpotin, column 12 lines 63-67. Kirpotin also teaches the encapsulation of doxorubicin (an anti-neoplastic agent) into a liposome at 129 nanomole per micromole, which is a ratio of at least about 0.10 moles of therapeutic entity to moles of lipid as of column 12 Example 1. Kirpotin teaches the use of PEG derivatized DSPE, as of column 9 lines 58-67, wherein said lipids comprise approximately 1/16 of the total lipids by mole.

Although Kirpotin does not specifically teach the functional characteristics of the liposome, the claimed liposome appears to be the same as the prior art. The office does

not have the facilities and resources to provide the factual evidence needed in order to establish that the product of the prior art does not possess the same material, structural and functional characteristics of the claimed product. In the absence of evidence to the contrary, the burden is on the applicant to prove that the claimed product is different from those taught by the prior art and to establish patentable differences. See In re Best 562F.2d 1252, 195 USPQ 430 (CCPA 1977) and Ex parte Gray 10 USPQ 2d 1922 (PTO Bd. Pat. App. & Int. 1989). Limitations deemed to be functional limitations comprise the limitations of claim 40 which recite that the liposome composition has in vivo anti-neoplastic activity at least four fold higher than the anti-neoplastic activity of the entity in a free non-liposomal form, and that of toxicity differences between the liposomal and non-liposomal forms. Other limitations deemed to be functional limitations include those of claims 34-36 and 39, which read on the percentage of drug encapsulated in a liposome after a period of time of storage (claims 34-35) and the percentage of drug in a liposome after administration to a rat (claims 36 and 39), and a longevity of less than two times higher of a liposome without PEG but identical in every other manner, as of claim 97.

The animal upon which the instantly claimed composition was tested, as of claim 98, simply expresses the intended use of the composition. Therefore, said limitations do not confer patentable weight to the instantly claimed composition. See MPEP 2111.04 (regarding "wherein" clauses), which states the following: “However, the court noted (quoting Minton v. Nat ’l Ass ’n of Securities Dealers, Inc., 336 F.3d 1373, 1381, 67 USPQ2d 1614, 1620 (Fed. Cir. 2003)) that a “whereby clause in a method claim is not

given weight when it simply expresses the intended result of a process step positively recited.'"

Claims 40-47 are rejected under 35 U.S.C. 102(b) as being anticipated by Sadzuka et al. (Cancer Letters 127, 1998, pp. 99-406).

The indefinite term "anti-neoplastic therapeutic entity," is examined under the art as if it reads on both the pro-drug and its active metabolite.

Sadzuka et al. (hereafter referred to as Sadzuka) teaches liposomes comprising lipids and CPT-11 on page 100 section 2.2 (starting bottom of left column). Said liposomes comprise DMPC/CH/DMPG at a 100:100:60 micromoles respectively of each lipid, as of Sadzuka, page 100, left column, bottom. Sadzuka also describes embodiments wherein DMPC is replaced by DSPC, and wherein DMPC or DSPC are coating with PEG (Sadzuka, page 100, right column, section 2.2). The examiner specifically points to the liposome used to test the side effects of CPT-11, which comprises 45 micromoles of CPT-11, as of Sadzuka, page 100 right column, last sentence of top paragraph. The trap ratio (encapsulation efficiency) of CPT-11 is at least 90%, as of Sadzuka, page 100 right column, first full paragraph. CPT-11 is equivalent to irinotecan, as of Sadzuka, page 99, abstract. Sadzuka teaches that liposomalization of CPT-11 suppresses CPT-11 induced diarrhea as lethal toxicity, as of page 105, left column, second full paragraph. Sadzuka teaches that when CPT-11 is encapsulated into a liposome comprising DSPC and PEG (S-PEG liposome), the concentrations of both the prodrug CPT-11 and its active metabolite SN-38, are both

over four-fold higher than that of CPT-11 administered free of a liposome, as of Sadzuka, page 103 left column, Figure 6B.

While Sadzuka does not explicitly teach a ratio of irinotecan to lipid that is at least 0.10, the claim limitation does not appear to result in a manipulative difference in the composition. A liposome comprising DMPC/CH/DMPG, at a 100:100:60 micromoles respectively of each lipid, comprises 260 micromoles of lipid. If 45 micromoles of CPT-11 were used at a 90% trap ratio, this results in 40.5 micromoles of CPT-11. The ratio of 40.5/260 is about 0.16, exceeding that of 0.1.

While Sadzuka does not explicitly teach that the toxicity of said irinotecan liposome, the claim limitation does not appear to result in a manipulative difference in the composition. Sadzuka which teaches that liposomalization of CPT-11 suppresses CPT-11 induced diarrhea as lethal toxicity, as of page 105, left column, second full paragraph.

While Sadzuka does not explicitly teach that the anti-neoplastic therapeutic activity of has an activity that is four times that of the non-liposomal form of said entity, the claim limitation does not appear to result in a manipulative difference in the composition. Sadzuka teaches that when CPT-11 is encapsulated into a liposome comprising DSPC and PEG (S-PEG liposome), the concentrations of both the prodrug CPT-11 and its active metabolite SN-38, are both over four-fold higher than that of CPT-11 administered free of a liposome, as of Sadzuka, page 103 left column, Figure 6B.

Although Sadzuka does not specifically teach the functional characteristics of the liposome, the claimed liposome appears to be the same as the prior art. The office does

not have the facilities and resources to provide the factual evidence needed in order to establish that the product of the prior art does not possess the same material, structural and functional characteristics of the claimed product. In the absence of evidence to the contrary, the burden is on the applicant to prove that the claimed product is different from those taught by the prior art and to establish patentable differences. See In re Best 562F.2d 1252, 195 USPQ 430 (CCPA 1977) and Ex parte Gray 10 USPQ 2d 1922 (PTO Bd. Pat. App. & Int. 1989). Limitations deemed to be functional limitations comprise the limitations of claim 41 which recite that the liposome composition is two or three times less toxic than the non-liposomal form.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 40-47 and 53-57 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rahman et al. (WO 03/030864 A1).

Rahman et al. (WO 03/030864 A1) (hereafter referred to as Rahman) teaches a liposomal composition comprising the active agent irinotecan which is used for treating cancer, as of the abstract of Rahman.

Rahman does not teach a ratio of the concentration of irinotecan to that of the lipid that is at least about 0.10, as of claim 40.

Rahman teaches that irinotecan is present in said composition at a ratio of “about 0.1 to 50, preferably of about 0.5 to 25, percent by weight of the total mixture,” as of Rahman page 6 lines 4-6. Hence, it would have been prima facie obvious for one of ordinary skill in that art to have utilized a composition wherein irinotecan is present at "at least about 0.10," as the range of Rahman overlaps with the range of at least about 0.10. Said overlap results in a prima facie case of obviousness, as of MPEP 2144.05(I).

"[A] prior art reference that discloses a range encompassing a somewhat narrower claimed range is sufficient to establish a prima facie case of obviousness." In re Peterson, 315 F.3d 1325, 1330, 65 USPQ2d 1379, 1382-83 (Fed. Cir. 2003).

Where a valid case of prima facie obviousness has been established, the burden shifts to applicant to demonstrate that a claimed functional property is applicable to the claim in its broad scope: In re Greenfield, 197 USPQ 227, 229 (CCPA 1978). (Holding that despite the fact that the rejection was one of obviousness and not anticipation, the burden was nevertheless on applicant to provide factual verification of the alleged functional property). Thus, even assuming *arguendo* that applicant has shown that a specific combination of iritotecan encapsulated into a liposome at a mole fraction of iritotecan to lipid of at least 0.1 might exhibit unexpected increase in in-vivo neoplastic activity (as of claim 40), lack of toxicity (as of claims 40-41), and half-release time (as of claims 46-47), this has not been shown for the broad genus of anti-neoplastic therapeutic entities (as of claims 40-43) and topoisomerase inhibitors (as of claims 44 and 46-47 and 55-57) currently claimed. The method upon which the instantly claimed composition was tested, as of claims 55-57, simply expresses the intended use of the composition. Therefore, said limitations do not confer patentable weight to the instantly claimed composition.

Claims 17-20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin (US Patent 6,110,491) as applied to claim 15 above, and further in view of

Nentwich (Intravenous Therapy, 1990) and Maddison et al. (Small Animal Clincal Pharmacology, 2002).

With regard to Maddison et al., the page number of the particular page shown as part of the record is not visible on the document that has been placed into the record. The examiner clarifies that the page displayed is page 437.

Kirpotin teaches a doxorubicin encapsulated liposome with a transmembrane gradient.

Kirpotin does not teach sucrose octasulfate.

Nentwich teaches that doxorubicin causes esophagitis as a side effect on page 310, right column, “Side Effects" section, "Gastrointestinal" subsection.

Kirpotin in view of Nentwich do not teach the use of sucrose octasulfate.

Maddison et al. (hereafter referred to as Maddison) teaches, on page 437, left column, section entitled “Clinical Applications,” that sucralfate is used to treat esophagitis. Maddison teaches that sucralfate is composed of sucrose octasulfate and aluminum hydroxide, which dissociate in the acid environment of the stomach, as of Maddison, page 437 left column, last paragraph, and page 437, right column, first full paragraph. The examiner interprets this to mean that sucralfate is a prodrug releasing sucrose octasulfate as an active ingredient.

It would have been prima facie obvious for one of ordinary skill in the art to have combined sucrose octasulfate with a liposomal formulation of doxorubicin. This is because the administration of doxorubicin for the treatment of cancer results in esophagitis, as of Nentwich, page 310. The esophagitis that occurs due to doxorubicin

therapy may be treated with sucrose octasulfate, as sucrose octasulfate is known to treat esophagitis, as of Maddison, page 437 left column, second to last paragraph.

Claims 96, 99, 102-113 and 119 are rejected under 35 U.S.C. 103(a) as being unpatentable over of Kirpotin (US Patent 6,110,491) as applied to claim 94 above, and further in view of Ahmad et al. (Cancer Research 53, 1484-1488, April 1, 1993).

Kirpotin teaches a liposome comprising doxorubicin as well as a transmembrane gradient of a polyanionic substance.

Kirpotin does not teach the use of pegylated lipids, wherein said PEG has a specific molecular weight. As of claims 103-104 and 112. Kirpotin further does not teach a targeting moiety, as of claims 105-113 and 119.

Ahmad et al. (hereafter referred to as Ahmad) teaches liposomes comprising doxorubicin, lipid containing derivatives of polyethylene glycol, and a targeting moiety wherein said targeting moiety is comprised of antibodies, as of Ahmad, page 1484, left column, abstract. The liposomes were prepared with PEG of a molecular weight of 1900 Daltons, (see Ahmad, page 1484, left column, third footnote, bottom of page), and were prepared with a molar ratio of HSPC:cholesterol:DSPE-PEG (wherein HSPC is phosphatidylcholine and DSPE-PEG is the lipid distearoylethanolamine that is bonded to PEG) that is 2:1:0.1, as of Ahmad, page 1484, right column, section entitled "Liposome Preparation." Said formulation results in a mole fraction of 1/31 (3.2%) of DSPE-PEG. Ahmad teaches that liposomes with PEG but in the absence of antibody results in approximately 37.5% of liposomes remaining in the blood after 24 hours, as

compared to undetectable amounts of liposomes remaining after 24 hours for those liposomes that lack PEG, as of Ahmad, page 1485, right column, last paragraph. Ahmad teaches animals injected with doxorubicin encapsulated into pegylated liposomes with an antibody experienced a greater reduction in lung tumor size as compared to animals injected with liposomes a targeting moiety, and that mice injected with liposomes comprising a targeting moiety survived longer than mice injected with liposomes lacking a targeting moiety, as of Ahmad, page 1487, left column, Table 1 and Figure 3.

It would have been prima facie obvious for one of ordinary skill in the art to have utilized PEG-1900 and a targeting moiety with the liposome made by the combination of Kirpotin. This is because the presence of PEG-1900 results in a longer half life of liposomal doxorubicin, as of Ahmad, page 1485, right column, last paragraph, resulting in greater tumor therapy. Furthermore, Kirpotin further teaches pegylation of lipids (wherein approximately 1-5% of lipids are pegylated) to increase circulation time and improve drug delivery, as of column 14 lines 13-20 and column 9 lines 58-67. As for the targeting moiety, Ahmad teaches that the presence of an antibody targeting moiety leads to significant tumor reduction and increase in survival rates, as of as of Ahmad, page 1487, left column, Table 1 and Figure 3. Therefore, one of ordinary skill in the art would have been motivated to use pegylated lipids, along with an antibody targeting moiety, to increase survivability, to more effectively treat tumors, and to increase the circulation half-life of the therapeutic agent.

Kirpotin view of Ahmad do not teach a liposome wherein pegylated lipids comprise less than 1% by mole of the total lipids, or between 0.1% to 0.9% by mole of the total lipids, as of claims 96 and 99.

It would have been prima facie obvious for one of ordinary skill in the art to have optimized the concentration of pegylated lipids in order to most effectively optimize the circulation half-life of said liposomes. The examiner points to MPEP 2144.05(II), which points out that optimization to discover the optimum or workable ranges by routine experimentation is not inventive. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." In re Aller, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

Claims 105-111, 113-116, and 119 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin (US Patent 6,110,491) as applied to claim 24 above, and further in view of Hong et al. (Annals of the New York Academy of Sciences, p. 293-296).

Kirpotin teaches a doxorubicin encapsulated pegylated liposome to treat cancer.

Kirpotin does not teach a targeting moiety, wherein said targeting moiety is a ErbB2 (HER-2) receptor.

Hong et al. (hereafter referred to as Hong), teaches that HER-2 is highly overexpressed in cancers, especially breast cancer, as of page 293, second paragraph.

Hong further teaches that the presence of an anti-HER2 targeting moiety in a doxorubicin immunoliposome (e.g. pegylated liposome) produced "marked antitumor effects," as of Hong, page 293, last paragraph.

It would have been prima facie obvious for one of ordinary skill in the art to have combined the pegylated, doxorubicin comprising liposomes of in view of Kirpotin with an ErbB2 targeting moiety, as said moiety would have yielded an improvement in cancer targeting, as of Hong, page 293, last paragraph. The formulations of Kirpotin and Hong use liposomal doxorubicin for the purposes of treating cancer, and the antibody of Hong would have improved the cancer targeting of the liposome of Kirpotin.

Claims 43-48 and 53-57 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin (US Patent 6,110,491) as applied to claims 13-16, 24-28, 30-32, 34-36, 39-42, 94-95, 97-98 and 100-101 and further in view of Rahman (WO 03/030864 A1).

Kirpotin discloses that liposomes loaded with the presence of polymeric anionic compounds such as polysaccharide sulfate (chondroitin sulfate, heparin etc.) (as of Kirpotin, column 5 lines 9-16) results in an increase of the concentration of encapsulated compound by several fold. Kirpotin teaches the presence of a gradient of charged polymer as of Kirpotin, column 9 lines 12-16. The examiner points to Kirpotin Example 1 (column 12 lines 5-36), which shows a doxorubicin ratio of 8 nmol/micromole for liposomes loaded without a polymeric sugar and in Example 3, (column 12 line 62 to column 13 lines 1-6, which also refers back to Example 1) a loading ratio of 26

nmol/micromole with the use of chondroitin sulfate. Said liposomes comprise 5 mg/mL (which is equivalent to grams per liter) of chondroitin sulfate in the inner buffer (i.e. liposome interior), as of Kirpotin, column 12 lines 63-67. Kirpotin also teaches the encapsulation of doxorubicin (an anti-neoplastic agent) into a liposome at 129 nanomole per micromole, which is a ratio of at least about 0.10 moles of therapeutic entity to moles of lipid as of column 12 Example 1.

Kirpotin does not teach the encapsulation of irinotecan.

Rahman et al. (WO 03/030864 A1) (hereafter referred to as Rahman) teaches a liposomal composition comprising the active agent irinotecan which is used for treating cancer, as of the abstract of Rahman. Rahman teaches liposome encapsulated irinotecan, as of page 5 lines 3-6 and other places. Rahman, page 1 lines 5-10 teaches that irinotecan treats cancer. Rahman also teaches, as of page 7 lines 1-8, that irinotecan reduces cancer cells to develop resistance to other agents like doxorubicin.

It would have been prima facie obvious for one of ordinary skill in the art to have combined liposomal irinotecan, as of Rahman, with doxorubicin, as of Kirpotin, as both drugs treat cancer. "It is prima facie obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their having been individually taught in the prior art." In re Kerkhoven, 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980) (citations omitted). Moreover, irinotecan reduces the tendency for cells to develop resistance to doxorubicin, making doxorubicin more effective so that the drugs work in tandem.

Rahman does not teach a ratio of the concentration of irinotecan to that of the lipid that is at least about 0.10, as of claim 40.

Rahman teaches that irinotecan is present in said composition at a ratio of "about 0.1 to 50, preferably of about 0.5 to 25, percent by weight of the total mixture," as of Rahman page 6 lines 4-6. Hence, it would have been prima facie obvious for one of ordinary skill in that art to have utilized a composition wherein irinotecan is present at "at least about 0.10," as the range of Rahman overlaps with the range of at least about 0.10. Said overlap results in a prima facie case of obviousness, as of MPEP 2144.05(I). "[A] prior art reference that discloses a range encompassing a somewhat narrower claimed range is sufficient to establish a prima facie case of obviousness." In re Peterson, 315 F.3d 1325, 1330, 65 USPQ2d 1379, 1382-83 (Fed. Cir. 2003).

Where a valid case of prima facie obviousness has been established, the burden shifts to applicant to demonstrate that a claimed functional property is applicable to the claim in its broad scope: In re Greenfield, 197 USPQ 227, 229 (CCPA 1978). (Holding that despite the fact that the rejection was one of obviousness and not anticipation, the burden was nevertheless on applicant to provide factual verification of the alleged functional property). Thus, even assuming *arguendo* that applicant has shown that a specific combination of iritotecan encapsulated into a liposome at a mole fraction of iritotecan to lipid of at least 0.1 might exhibit unexpected increase in in-vivo neoplastic activity (as of claim 40), lack of toxicity (as of claims 40-41), and half-release time (as of claims 46-47), this has not been shown for the broad genus of anti-neoplastic

therapeutic entities (as of claims 40-43) and topoisomerase inhibitors (as of claims 44 and 46-47 and 55-57) currently claimed.

Claims 49-50 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin (US Patent 6,110,491) in view of Rahman (WO 03/030864 A1) as applied to claim 44 above, and further in view of Shimada et al. (Surg. Today, 2002, 32:1075-1080) and Maddison et al. (Small Animal Clincal Pharmacology, 2002).

With regard to Maddison et al., the page number of the particular page shown as part of the record is not visible on the document that has been placed into the record. The examiner clarifies that the page displayed is page 437.

Kirpotin in view of Rahman et al. (hereafter referred to as Rahman) teach a liposomal composition comprising the active agent irinotecan which is used for treating cancer, as of the abstract of Rahman, wherein said liposome is loaded with the presence of polymeric anionic compounds such as polysaccharide sulfate (chondroitin sulfate, heparin etc.) (as of Kirpotin, column 5 lines 9-16). This results in an increase of the concentration of encapsulated compound by several fold, as of Kirpotin (Example 2, column 12 lines 37-60). Kirpotin teaches the presence of a gradient of charged polymer as of Kirpotin, column 9 lines 12-16.

Kirpotin in view of Rahman do not teach the incorporation of liposomal irinotecan with a polyanion, wherein said polyanion is sucrose octasulfate.

Shimada et al. (hereafter referred to as Shimada), teaches that an ulcer is formed following treatment with CPT-11 (irinotecan), as of Shimada, pages 1076-1078, case reports for patients 1 and 2. The examiner specifically points out Figures 1B and 5B, which show, that for patients 1 and 2 respectively, chemotherapy with irinotecan results in the formation of an ulcer following treatment. The examiner further points to Shimada, page 1077, left column, bottom of page, which shows the formation of an ulcer as to Patient 1, and Shimada page 1078, left column, first paragraph (above *Chemotherapy Regimen and Evaluation of Response*), which shows the formation of an ulcer upon treatment with CPT-11. Shimada, page 1075, right column, first full paragraph, shows the equivalency between the terms "CPT-11" and irinotecan. The treatment of Shimada is being used for gastric carcinomas, as of Shimada, page 1075 left column, abstract.

Kirpotin in view of Rahman and further in view of Shimada do not teach the incorporation of liposomal irinotecan with a polyanion, wherein said polyanion is sucrose octasulfate.

Maddison et al. (hereafter referred to as Maddison) teaches that the drug sucralfate is known for the treatment of gastric ulceration, as of Maddison, page 437 left column, second to last paragraph. Maddison teaches that sucralfate is composed of sucrose octasulfate and aluminum hydroxide, which dissociate in the acid environment of the stomach, as of Maddison, page 437 left column, last paragraph, and page 437, right column, first full paragraph. The examiner interprets this to mean that sucralfate is a prodrug releasing sucrose octasulfate as an active ingredient.

It would have been prima facie obvious for one of ordinary skill in the art to have combined sucrose octasulfate with a liposomal formulation of irinotecan. This is because the administration of irinotecan for the treatment of gastric cancers results in the formation of ulcers, as of Shimada, pages 1077-1078, reports for Patients 1 and 2. The ulcers formed due to irinotecan therapy may be treated with sucrose octasulfate, as sucrose octasulfate is known to treat stomach ulcers, as of Maddison, page 437 left column, second to last paragraph. It further would have been prima facie obvious for one of ordinary skill in the art to have encapsulated irinotecan into a liposome, as a liposomal formulation results in avoidance of solubility problems, reduced drug toxicity, increased therapeutic efficacy of the drug, and modulation of multidrug resistance, as of Shimada, page 2 lines 30-37.

Claims 52 and 161 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin (US Patent 6,110,491) in view of Rahman (WO 03/030864 A1) as applied to claim 44 above, and further in view of Lee et al. (Cancer Research, 62, August 1, 2002, 4282-4288).

Kirpotin in view of Rahman et al. (hereafter referred to as Rahman) teach a liposomal composition comprising the active agent irinotecan which is used for treating cancer, as of the abstract of Rahman, wherein said liposome is loaded with the presence of polymeric anionic compounds such as polysaccharide sulfate (chondroitin sulfate, heparin etc.) (as of Kirpotin, column 5 lines 9-16). This results in an increase of

the concentration of encapsulated compound by several fold, as of Kirpotin (Example 2, column 12 lines 37-60). Kirpotin teaches the presence of a gradient of charged polymer as of Kirpotin, column 9 lines 12-16.

Kirpotin in view of Rahman do not teach the presence of the triethylammonium ion, as of claims 52 and 161.

Lee et al. (hereafter referred to as Lee) teaches the encapsulation of rhodamine B-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine triethylammonium salt (rhodamine-PE). The applicant is instructed to note page 4283 left column, section entitled “Liposome and Liposomal Cisplatin Preparation,” wherein the loading of rhodamine-PE is described, as well as page 4282, left column, third footnote, wherein it is shown that the abbreviation rhodamine-PE describes rhodamine B-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine triethylammonium salt. Lee shows that rhodamine-PE is used to measure the uptake of anti-tumor agent cisplatin-containing liposomes in tumor cells in vitro (see, for example, page 4284 right column Figure 3, and the section entitled "Cisplatin Entrapped in TRX-20 Liposomes but not in Plain PEG Liposomes Kills CS-expressing Tumor Cells *in Vitro*, found on page 4284 right column, directly above Figure 3). Rhodamine-PE was also used to test biodistribution of anti-cancer liposomes in tumor-bearing mice (see, for example, page 4283 right column, section entitled "Biodistribution of TRX-20 Liposomes in s.c. Tumor-bearing Mice," as well as page 4285, left column, Figure 5, which shows the results obtained from such an experiment).

It would have been prima facie obvious for one of ordinary skill in the art to have added rhodamine-PE to the irinotecan-comprising liposomes of Kirpotin in view of Rahman. Lee teaches the use of rhodamine-PE to examine the properties of liposomes comprising an anti-tumor agent both in vitro (see, for example, page 4284 right column Figure 3, and the section entitled "Cisplatin Entrapped in TRX-20 Liposomes but not in Plain PEG Liposomes Kills CS-expressing Tumor Cells *in Vitro*, found on page 4284 right column, directly above Figure 3) and in vivo (see, for example, page 4283 right column, section entitled "Biodistribution of TRX-20 Liposomes in s.c. Tumor-bearing Mice," as well as page 4285, left column, Figure 5, which shows the results obtained from such an experiment). As the liposomes of Rahman comprise an anti-tumor agent (see the abstract of Rahman), one of ordinary skill in the art at the time the invention was made would have been motivated to add rhodamine-PE to the anti-cancer liposomes of Rahman to test their properties with regard to tumor targeting both in vitro and in vivo.

Claims 156-158, 162, and 164 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin (US Patent 6,110,491) as applied to claim 15 above, and further in view of Hope et al. (US Patent 5,785,987).

Kirpotin (US Patent 6,110,491) (hereafter referred to as Kirpotin) teaches liposomes comprising doxorubicin and a transmembrane ion concentration gradient of polyanionic polyols.

Kirpotin does not teach a kit as of claims 156-157. Kirpotin does not teach substituted ammonium ions, as of claims 158, 162, and 164.

Hope et al. (US Patent 5,785,987) (hereafter referred to as Hope) teaches the use of ethanolammonium dichloride gradient to load anti-cancer drugs into a liposome, as of Hope (column 17 line 47-column 18 line 14 Example 2). Hope further teaches gradients of ethylenediammonium sulfate (column 18 lines 15-20) and methylammonium sulfate (column 18 lines 52-56). Hope teaches the loading of said liposomes with a variety of active agents such as anti-cancer agents and antibioitics (column 12 lines 8-27 and column 13 lines 17-35). Hope specifically teaches the encapsulation of Doxorubicin, as of Figure 3 and Example 2 (column 17 line 47-column 18 line 14). Hope (column 11 lines 20-28) teaches that the concentration of the methylammonium salt which is encapsulated varies from 50 mM to 1M. Hope, teaches pegylation of lipids (wherein approximately 1-5% of lipids are pegylated) to increase circulation time and improve drug delivery (column 14 lines 13-20). Hope further teaches the presence of the liposome in a kit, and therapeutic agents in a separate kit, such that they can be mixed together prior to use, wherein said kit comprises instructions for encapsulation of the active agent and its use, as of Hope, column 14 lines 39-60.

It would have been prima facie obvious for one of ordinary skill in the art to have combined a substituted ammonium ion gradient of Hope with the liposomes of Kirpotin, as said gradient may be used to load liposomes with anti-cancer drugs, as of Hope (column 17 line 47-column 18 line 14 Example 2). Kirpotin teaches that polyanions are

used to form a gradient to load anti-cancer drugs, as of Kirpotin, column 9 lines 12-16 (which shows gradients of polyanions) and Kirpotin, examples, which show an anti-cancer drug. Hence, both the ammonium ion gradients and polyanions serve the same purpose in that they increase the amount of doxorubicin loaded into liposomes, and a case of prima facie obviousness is established. "It is prima facie obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their having been individually taught in the prior art." In re Kerkhoven, 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980) (citations omitted).

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ISAAC SHOMER whose telephone number is (571)270-7671. The examiner can normally be reached on 8:00 AM - 5:00 PM Monday-Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Frederick F. Krass can be reached on (571)272-0580. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/I. S./
Examiner, Art Unit 1612

/Brandon J Fetterolf/

Primary Examiner, Art Unit 1642

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EXAMINER	
SHOMER, ISAAC	

ART UNIT	PAPER NUMBER
1612	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	11/121,294	HONG ET AL.	
	Examiner	Art Unit	
	ISAAC SHOMER	1612	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *19 January 2010*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *13-19,21,22,24-42,44-51,53-58,61-66 and 68-164* is/are pending in the application.

4a) Of the above claim(s) *See Continuation Sheet* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *13-19,21,22, 24-28, 30-32, 34-36, 39-42, 44-50, 53-57, 94-116, 119, 156-158, 161, 162, and 164* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *22 October 2009*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Continuation of Disposition of Claims: Claims withdrawn from consideration are 29,33,37,38,51,58,61-66,68-93,117,118,120-155,159,160 and 163.

DETAILED ACTION

Applicants' arguments, filed 19 January 2010, have been fully considered. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn. The following rejections and/or objections are either reiterated or newly applied. They constitute the complete set presently being applied to the instant application.

Claim Rejections - 35 USC § 103

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 13, 14, 16-19, 21, 22, 24-28, 30-32, 34-36, 39-42, 94, 95, 97, 98, 100, 101, 156-158, 161, 162, and 164 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin (US Patent 6,110,491) in view of Hope et al. (US Patent 5,785,987) and Nentwich (Intravenous Therapy, 1990) and Maddison et al. (Small Animal Clinical Pharmacology, 2002).

Kirpotin teaches liposomes loaded with a drug such as doxorubicin in the presence of a polyanionic sugar such as chondroitin sulfate. Kirpotin teaches the presence of a gradient of charged polymer as of Kirpotin, column 9 lines 12-16. The examiner points to Kirpotin Example 1 (column 12 lines 5-36), which shows a doxorubicin ratio of 8 nmol/micromole for liposomes loaded without a polymeric sugar and in Example 3, (column 12 line 62 to column 13 lines 1-6, which also refers back to

Example 1) a loading ratio of 26 nmol/micromole with the use of chondroitin sulfate. Said liposomes comprise 5 mg/mL (which is equivalent to grams per liter) of chondroitin sulfate in the inner buffer (i.e. liposome interior), as of Kirpotin, column 12 lines 63-67. Kirpotin also teaches the encapsulation of doxorubicin (an anti-neoplastic agent) into a liposome at 129 nanomole per micromole, which is a ratio of at least about 0.10 moles of therapeutic entity to moles of lipid as of column 12 Example 1. Kirpotin teaches the use of PEG derivatized DSPE, as of column 9 lines 58-67, wherein said lipids comprise approximately 1/16 of the total lipids by mole.

Kirpotin does not teach the anion of a polyanionized monosaccharide or a polyanionized disaccharide. Kirpotin further does not teach the transmembrane gradient of ammonium ions.

Hope et al. (hereafter referred to as Hope) teaches the use of ethanolammonium chloride gradient, as well as a methylammonium gradient to load anti-cancer drugs into a liposome, as of Hope, column 17 line 47 to column 18 line 14, Example 3. See examiner's action, page 29, second full paragraph. Hope further teaches gradients of ethylenediammonium sulfate (column 18 lines 15-20) and methylammonium sulfate (column 18 lines 52-56). Hope teaches the loading of said liposomes with a variety of active agents such as anti-cancer agents and antibioitics (column 12 lines 8-27 and column 13 lines 17-35). Hope specifically teaches the encapsulation of Doxorubicin, as of Figure 3 and Example 2 (column 17 line 47-column 18 line 14). Hope (column 11 lines 20-28) teaches that the concentration of the methylammonium salt which is encapsulated varies from 50 mM to 1M. Hope, teaches pegylation of lipids (wherein

approximately 1-5% of lipids are pegylated) to increase circulation time and improve drug delivery (column 14 lines 13-20). Hope further teaches the presence of the liposome in a kit, and therapeutic agents in a separate kit, such that they can be mixed together prior to use, wherein said kit comprises instructions for encapsulation of the active agent and its use, as of Hope, column 14 lines 39-60.

It would have been prima facie obvious for one of ordinary skill in the art to have combined the ammonium ion gradient of Hope with the liposome comprising an anionic sugar of Kirpotin, as both are used to load anti-cancer drugs into a liposome. See examiner's action, paragraph bridging pages 29 and 30. Generally, it is *prima facie* obvious to combine two compositions, each of which is taught by the prior art to be useful for same purpose, in order to form a third composition to be used for the very same purpose. The idea for combining them flows logically from their having been individually taught in the prior art. See MPEP 2144.06.

Neither Kirpotin nor Hope teach a polyanionized monosaccharide or disaccharide.

Nentwich teaches that doxorubicin causes esophagitis as a side effect on page 310, right column, "Side Effects" section, "Gastrointestinal" subsection.

Maddison et al. (hereafter referred to as Maddison) teaches that sucralfate, which is a combination of sucrose octasulfate and aluminum hydroxide, is used to treat esophagitis. See examiner's action, page 17, last full paragraph.

It would have been prima facie obvious for one of ordinary skill in the art to have combined sucrose octasulfate with a liposomal form of doxorubicin, as sucrose

octasulfate treats esophagitis, which is a side effect of the administered drug. See examiner's action, paragraph bridging pages 17 and 18.

In regards to the combination of Kirpotin with Hope, applicant argues in applicant's arguments dated 19 January 2010 (hereafter referred to as applicant's arguments) that one of ordinary skill in the art would not have been motivated to have combined the teachings of Kirpotin and Hope, as Hope teaches that drug retention in large unilamellar vesicles was not improved by the use of alkylammonium sulfates as compared to ammonium by itself, as of applicant's arguments, paragraph bridging pages 46 and 47. No citation in Hope is given for this assertion. Applicant further argues that neither Kirpotin nor Hope teach a composition comprising a polyanionized monosaccharide or disaccharide, as of applicant's arguments, page 47 first full paragraph and paragraph bridging pages 47 and 48.

In response, the examiner notes that Hope teaches that the use of methylammonium ion gradients enable the rapid loading of drugs such as ciprofloxacin and result in broader loading possibilities (as compared to ammonium ion), as of Hope, column 10 lines 54-60. As such, Hope does appear to teach the benefits of using a methylammonium ion gradient, contrary to applicant's contention otherwise. If, purely *en arguendo*, Hope teaches that methylammonium ion gradient has no advantages over an ammonium sulfate gradient, as asserted by applicant, one of ordinary skill in the art would still have been motivated to have used a methylammonium ion gradient as it would have predictably been suitable for the intended use of a liposome gradient. The use of a known technique (i.e. methylammonium ions as a liposome gradient) to

improve a known product (e.g. a liposome) is prima facie obvious. See MPEP 2143, Exemplary Rationale D.

In regards to the combination of Kirpotin with Nentwich and Maddison, applicant argues that Nentwich does not cure the deficiencies of Kirpotin as it does not teach or suggest a polyanionized monosaccharide or disaccharide, as of applicant's arguments, page 32, second full paragraph. Applicant further argues that Maddison does not cure the defects of Kirpotin or Nentwich because it teaches sucrasulfate, a "combination of sucrose octasulfate and aluminum hydroxide." (paragraph bridging pages 32 and 33). Despite this admission, applicant asserts that neither Maddison, Kirpotin, or Nentwich do not teach the claimed invention, as of applicant's arguments, page 33, both paragraphs.

In response, the examiner notes that Maddison does teach sucrose octasulfate, as admitted by applicant on page 33, first line of applicant's arguments. Despite the fact that sucrose octasulfate is combined with aluminum hydroxide, the combination of references still teaches sucrose octasulfate due to the inclusive interpretation of the term "comprising" as an open-ended term which does not exclude additional unrecited elements. See MPEP 2111.03, second full paragraph.

Claim 15 is rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin (US Patent 6,110,491) in view of Hope et al. (US Patent 5,785,987) and Nentwich (Intravenous Therapy, 1990) and Maddison et al. (Small Animal Clincal Pharmacology,

2002) as applied to claims 13, 14, 16-19, 21, 22, 24-28, 30-32, 34-36, 39-42, 94, 95, 97, 98, 100, 101, 156-158, 162, and 164 above, and further in view of Katsu et al. (Anal. Chem., 2001, Vol. 73, pp. 1849-1854).

Kirpotin in view of Hope, Nentwich, and Maddison teach a liposome comprising ethanolammonium chloride gradient, as well as a methylammonium gradient, as shown above.

The references above do not teach a dialkyl or trialkyl ammonium ion.

Katsu et al. (hereafter referred to as Katsu) teaches a triethylamine and triethylammonium ion gradient as a pH gradient, as of Katsu, page 1850 left column, equation (2) and first full paragraph. Said gradient is across a liposome, as of Katsu, page 1852, right column, first full paragraph.

It would have been prima facie obvious for one of ordinary skill in the art to have substituted a triethylammonium ion gradient, as of Katsu, for the methylammonium ion gradient in the liposomes of Kirpotin in view of Hope, Nentwich, and Maddison. This is because triethylammonium ion is a known to be suitable for the intended use of a gradient for loading liposomes. As such, one of ordinary skill in the art would have been motivated to have substituted triethylammonium ions for methylammonium ions with a predictable expectation of successful use in a liposome gradient. The simple substitution of one known element for another to obtain predictable results is prima facie obvious. See MPEP 2143, Exemplary Rationale B.

Claims 44-50 and 53-57 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin (US Patent 6,110,491) in view of Hope et al. (US Patent 5,785,987) and Nentwich (Intravenous Therapy, 1990) and Maddison et al. (Small Animal Clincal Pharmacology, 2002) as applied to claims 13, 14, 16-19, 21, 22, 24-28, 30-32, 34-36, 39-42, 94, 95, 97, 98, 100, 101, 156-158, 162, and 164 above, and further in view of Rahman (WO 03/030864 A1).

Kirpotin in view of Hope, Nentwich, and Maddison teach a liposome comprising ethanolammonium chloride gradient, as well as a methylammonium gradient, as shown above.

The references above do not teach irinotecan.

Rahman et al. (WO 03/030864 A1) (hereafter referred to as Rahman) teaches a liposomal composition comprising the active agent irinotecan which is used for treating cancer, as of the abstract of Rahman. Rahman teaches liposome encapsulated irinotecan, as of page 5 lines 3-6 and other places. Rahman, page 1 lines 5-10 teaches that irinotecan treats cancer. Rahman also teaches, as of page 7 lines 1-8, that irinotecan reduces cancer cells to develop resistance to other agents like doxorubicin.

It would have been prima facie obvious for one of ordinary skill in the art to have combined liposomal irinotecan, as of Rahman, with doxorubicin, as of Kirpotin, as both drugs treat cancer. Generally, it is *prima facie* obvious to combine two compositions, each of which is taught by the prior art to be useful for same purpose, in order to form a third composition to be used for the very same purpose. The idea for combining them

flows logically from their having been individually taught in the prior art. See MPEP 2144.06.

Claims 96, 99, 102-113 and 119 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin (US Patent 6,110,491) in view of Hope et al. (US Patent 5,785,987) and Nentwich (Intravenous Therapy, 1990) and Maddison et al. (Small Animal Clincal Pharmacology, 2002) as applied to claims 13, 14, 16-19, 21, 22, 24-28, 30-32, 34-36, 39-42, 94, 95, 97, 98, 100, 101, 156-158, 162, and 164 above, and further in view of Ahmad et al. (Cancer Research, 53, pp. 1484-1488, April 1, 1993).

Kirpotin in view of Hope, Nentwich, and Maddison teach a liposome comprising ethanolammonium chloride gradient, as well as a methylammonium gradient, as shown above.

The references above do not teach a liposome comprising doxorubicin and pegylated lipids wherein PEG has a specific molecular weight. The claims above do not teach a targeting moiety.

Ahmad et al. (hereafter referred to as Ahmad) teaches liposomes comprising doxorubicin, lipid containing derivatives of polyethylene glycol, and a targeting moiety wherein said targeting moiety is comprised of antibodies, as of Ahmad, page 1484, left column, abstract. The liposomes were prepared with PEG of a molecular weight of 1900 Daltons, (see Ahmad, page 1484, left column, third footnote, bottom of page), and were prepared with a molar ratio of HSPC:cholesterol:DSPE-PEG (wherein HSPC is

phosphatidylcholine and DSPE-PEG is the lipid distearoylethanolamine that is bonded to PEG) that is 2:1:0.1, as of Ahmad, page 1484, right column, section entitled "Liposome Preparation." Said formulation results in a mole fraction of 1/31 (3.2%) of DSPE-PEG. Ahmad teaches that liposomes with PEG but in the absence of antibody results in approximately 37.5% of liposomes remaining in the blood after 24 hours, as compared to undetectable amounts of liposomes remaining after 24 hours for those liposomes that lack PEG, as of Ahmad, page 1485, right column, last paragraph. Ahmad teaches animals injected with doxorubicin encapsulated into pegylated liposomes with an antibody experienced a greater reduction in lung tumor size as compared to animals injected with liposomes a targeting moiety, and that mice injected with liposomes comprising a targeting moiety survived longer than mice injected with liposomes lacking a targeting moiety, as of Ahmad, page 1487, left column, Table 1 and Figure 3.

It would have been prima facie obvious for one of ordinary skill in the art to have utilized PEG-1900 and a targeting moiety with the liposome made by the combination of Kirpotin. This is because the presence of PEG-1900 results in a longer half life of liposomal doxorubicin, as of Ahmad, page 1485, right column, last paragraph, resulting in greater tumor therapy. Furthermore, Kirpotin further teaches pegylation of lipids (wherein approximately 1-5% of lipids are pegylated) to increase circulation time and improve drug delivery, as of column 14 lines 13-20 and column 9 lines 58-67. As for the targeting moiety, Ahmad teaches that the presence of an antibody targeting moiety leads to significant tumor reduction and increase in survival rates, as of as of Ahmad,

page 1487, left column, Table 1 and Figure 3. Therefore, one of ordinary skill in the art would have been motivated to use pegylated lipids, along with an antibody targeting moiety, to increase survivability, to more effectively treat tumors, and to increase the circulation half-life of the therapeutic agent

Claims 105-111, 113-116 and 119 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin (US Patent 6,110,491) in view of Hope et al. (US Patent 5,785,987) and Nentwich (Intravenous Therapy, 1990) and Maddison et al. (Small Animal Clincal Pharmacology, 2002) as applied to claims 13, 14, 16-19, 21, 22, 24-28, 30-32, 34-36, 39-42, 94, 95, 97, 98, 100, 101, 156-158, 162, and 164 above, and further in view of Hong et al. (Annals of the New York Academy of Sciences, pp. 293-296).

Kirpotin in view of Hope, Nentwich, and Maddison teach a liposome comprising ethanolammonium chloride gradient, as well as a methylammonium gradient, as shown above.

The claims above do not teach a targeting moiety, wherein said moiety is a ErbB2 (HER-2) receptor.

Hong et al. (hereafter referred to as Hong), teaches that HER-2 is highly overexpressed in cancers, especially breast cancer, as of page 293, second paragraph. Hong further teaches that the presence of an anti-HER2 targeting moiety in a doxorubicin immunoliposome (e.g. pegylated liposome) produced "marked antitumor effects," as of Hong, page 293, last paragraph.

It would have been prima facie obvious for one of ordinary skill in the art to have combined the pegylated, doxorubicin comprising liposomes of in view of Kirpotin with an ErbB2 targeting moiety, as said moiety would have yielded an improvement in cancer targeting, as of Hong, page 293, last paragraph. The formulations of Kirpotin and Hong use liposomal doxorubicin for the purposes of treating cancer, and the antibody of Hong would have improved the cancer targeting of the liposome of Kirpotin.

Conclusion

No claim is allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ISAAC SHOMER whose telephone number is (571)270-7671. The examiner can normally be reached on 8:00 AM - 5:00 PM Monday-Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Frederick F. Krass can be reached on (571)272-0580. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/I. S./
Examiner, Art Unit 1612

/Frederick Krass/
Supervisory Patent Examiner, Art Unit 1612

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/121,294	05/02/2005	Keelung Hong	HERM1130-1	2502

28213 7590 05/19/2010

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EXAMINER	
SHOMER, ISAAC	

ART UNIT	PAPER NUMBER
1612	

MAIL DATE	DELIVERY MODE
05/19/2010	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Advisory Action Before the Filing of an Appeal Brief	**Application No.** 11/121,294	**Applicant(s)** HONG ET AL.	
	Examiner ISAAC SHOMER	**Art Unit** 1612	

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 11 May 2010 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

1. ☒ The reply was filed after a final rejection, but prior to or on the same day as filing a Notice of Appeal. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance; (2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114. The reply must be filed within one of the following time periods:
   a) ☐ The period for reply expires _____months from the mailing date of the final rejection.
   b) ☒ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.

   Examiner Note: If box 1 is checked, check either box (a) or (b). ONLY CHECK BOX (b) WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☐ The Notice of Appeal was filed on _____. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37 CFR 41.37(a).

AMENDMENTS

3. ☒ The proposed amendment(s) filed after a final rejection, but prior to the date of filing a brief, will not be entered because
   (a) ☒ They raise new issues that would require further consideration and/or search (see NOTE below);
   (b) ☐ They raise the issue of new matter (see NOTE below);
   (c) ☒ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
   (d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.

   NOTE: *See Continuation Sheet*. (See 37 CFR 1.116 and 41.33(a)).

4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).
5. ☐ Applicant's reply has overcome the following rejection(s): _____.
6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).
7. ☒ For purposes of appeal, the proposed amendment(s): a) ☒ will not be entered, or b) ☐ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.
   The status of the claim(s) is (or will be) as follows:
   Claim(s) allowed: _____.
   Claim(s) objected to: _____.
   Claim(s) rejected: *13-19,21,22,24-28,30-32,34-36,39-42,44-50,53-57,94-116,119,156-158,161,162 and 164*.
   Claim(s) withdrawn from consideration: *29,33,37,38,51,58,61-66,68-93,117,118,120-155,159,160,163*.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ The affidavit or other evidence filed after a final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).
9. ☐ The affidavit or other evidence filed after the date of filing a Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing a good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).
10. ☐ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

11. ☒ The request for reconsideration has been considered but does NOT place the application in condition for allowance because: See Continuation Sheet.
12. ☐ Note the attached Information *Disclosure Statement*(s). (PTO/SB/08) Paper No(s). _____
13. ☐ Other: _____.

/Frederick Krass/
Supervisory Patent Examiner, Art Unit 1612

/I. S./
Examiner, Art Unit 1612

Continuation of 3. NOTE: Applicant has proposed to amend claim 13 to recite the limitation "a pharmaceutical formulation for parenteral administration". This limitation was not previously considered in regard to the compositions of claims 13 and all claims dependent upon claim 13, and further analysis would be required to determine whether utility of the prior art composition for parenteral administration would have been been obvious. Additionally a further search would also have to be made to determine the state of the art with regard to this issue. .

Continuation of 11. does NOT place the application in condition for allowance because: Applicant's arguments that the newly amended claims are patentable over the prior art references are moot at this time due to non-entry of the proposed amendment. .

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11/121,294	05/02/2005	Keelung Hong	HERM1130-1	2502

28213 7590 08/04/2010

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EXAMINER	
SHOMER, ISAAC	

ART UNIT	PAPER NUMBER
1612	

MAIL DATE	DELIVERY MODE
08/04/2010	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	11/121,294	HONG ET AL.	
	Examiner	Art Unit	
	ISAAC SHOMER	1612	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *27 May 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *13-19,21,22,25-42,44-51,53-58,61-66,68-155 and 158-164* is/are pending in the application.

4a) Of the above claim(s) *See Continuation Sheet* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *13-19,21,22,25-28,30-32,34-36,39-42,44-50,53-57,94-116,119,158,161,162 and 164* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *27 May 2010*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Continuation of Disposition of Claims: Claims withdrawn from consideration are 29,33,37,38,51,58,61-66,68-93,117,118,120-155,159,160 and 163.

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 27 May 2010 has been entered.

DETAILED ACTION

Applicants' arguments, filed 11 May 2010, have been fully considered. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn. The following rejections and/or objections are either reiterated or newly applied. They constitute the complete set presently being applied to the instant application.

Claim Rejections - 35 USC § 103

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 13, 16-19, 21, 22, 25-28, 30-32, 34-36, 39-42, 94, 95, 97, 98, 100, 101, 158, 161, 162, and 164 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin et al. (US Patent 6,110,491) in view of Koch et al. (US Patent 5,538,954).

Kirpotin et al. (hereafter referred to as Kirpotin) teaches a liposome composition comprising an encapsulated [pharmaceutically active] compound in a stable precipitated form, wherein the concentration of said active compound is higher in the interior space of the liposome than in the medium surrounding the liposome, as of Kirpotin, abstract. The interior space of the liposome of Kirpotin includes an ionizable active compound and a charged precipitating agent (as of Kirpotin, column 2 lines 40-43), where the precipitating agent has a charge opposite to that of the ionizable active compound, as of Kirpotin, column 3 lines 24-27. In one example, the precipitating agent is a charged polymer, wherein said polymer has a molecular weight of 400 to 2 million daltons, as of Kirpotin, column 4 line 61 to column 5 line 8. Kirpotin suggests a compound that ionizes to a positive charge, and a precipitating agent that is a multivatlent acid such as a polysulfate, as of Kirpotin, column 4 lines 20-29. In a more specific example, the active compound is doxorubicin which is cationic and the precipitating agent is an anion of phosphate, sulfate, citrate, polyacrylate, or other anions, as of Kirpotin, column 11 lines 55-58. Kirpotin also teaches the use of a pH or electrochemical ion gradient, wherein said gradient has the same charge as the compound to be loaded, as of Kirpotin, column 3 lines 23-31. Ammonium ion gradients are suggested as of Kirpotin, Example 6, column 13 line 64. Parenteral administration is suggested as of Kirpotin, column 8 lines 39-40. In one example, Kirpotin teaches a doxorubicin ratio of 8 nmol/micromole for liposomes loaded without a polymeric sugar (as of Kirpotin Example 1 (column 12 lines 5-36)) and in a loading ratio of 26 nmol/micromole with the use of the anionic polymeric precipitating agent chondroitin sulfate (as of Kirpotin Example 3, (column 12

line 62 to column 13 lines 1-6, which also refers back to Example 1)). Said liposomes with precipitating agent comprise 5 mg/mL (which is equivalent to grams per liter) of chondroitin sulfate in the inner buffer (i.e. liposome interior), as of Kirpotin, column 12 lines 63-67. Kirpotin teaches the encapsulation of doxorubicin (an anti-cancer agent) into a liposome at 129 nanomole per micromole, which is a ratio of at least about 0.10 moles of therapeutic entity to moles of lipid as of Kirpotin, column 12 Example 1. Kirpotin teaches the use of PEG derivatized DSPE (pegylated DSPE), as of column 9 lines 58-67, wherein said pegylated lipids comprise approximately 1/16 of the total lipids by mole.

Kirpotin does not teach sucrose octasulfate (the elected specie of precipitating agent).

Koch et al. (hereafter referred to as Koch) teaches a salt of doxycycline (a cation) wherein sucrose octasulfate (an anion) is the counter-ion, as of Koch, column 5 lines 7-16.

It would have been prima facie obvious for one of ordinary skill in the art to have used sucrose octasulfate as the anionic precipitating agent, as of Koch, in the liposome of Kirpotin. This is because sucrose octasulfate is known to predictably act as an anionic counter-ion for a cationic active agent with a reasonable expectation of success, as taught by Koch above. Furthermore, Kirpotin teaches that the precipitating agnet serves as the counter-ion to the charged drug, as of Kirpotin, column 4 lines 62-67. Sucrose octasulfate is chemically within the genus of a polysulfate (as suggested by Kirpotin, column 4 line 24) and in the genus of anionic polysaccharides used as the

charged polymer precipitating agent, as of Kirpotin, column 4 line 61 to column 5 line 21 (note the molecular weight range on column 5 line 4), and as such would have predictably acted as a precipitating agent with a reasonable expectation of success. Generally, it is *prima facie* obvious to select a known material for incorporation into a composition, based on its recognized suitability for its intended use. See MPEP 2144.07.

Claims 14 and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin et al. (US Patent 6,110,491) in view of Koch et al. (US Patent 5,538,954) as applied to claims 13, 16-19, 21, 22, 25-28, 30-32, 34-36, 39-42, 94, 95, 97, 98, 100, 101,156-158, 161,162, and 164 above, and further in view of Katsu et al. (Anal Chem., 2001, Vol. 73, pp. 1849-1854).

Kirpotin in view of Koch teaches a liposome comprising an active agent and a polymer with the opposite charge, wherein said polymer may be sucrose octasulfate. See the above rejection. Kirpotin teaches ammonium ion gradients are suggested as of Kirpotin, Example 6, column 13 line 64. Kirpotin also teaches the use of a pH or electrochemical ion gradient, wherein said gradient has the same charge as the compound to be loaded, as of Kirpotin, column 3 lines 23-31.

The above references do not teach a dialkyl or trialkyl ammonium ion gradient.

Katsu et al. (hereafter referred to as Katsu) teaches a triethylamine and triethylammonium ion gradient as a pH gradient, as of Katsu, page 1850 left column,

equation (2) and first full paragraph. Said gradient is across a liposome, as of Katsu, page 1852, right column, first full paragraph.

It would have been prima facie obvious for one of ordinary skill in the art to have used a triethylammonium ion gradient, as of Katsu, for the ammonium ion gradient in the liposomes of the above references. This is because triethylammonium ion is a known to be suitable for the intended use of a gradient for loading liposomes. As such, one of ordinary skill in the art would have been motivated to have substituted triethylammonium ions for methylammonium ions to have predictably created an ion gradient into a liposome with a reasonable expectation of success. Generally, it is *prima facie* obvious to select a known material for incorporation into a composition, based on its recognized suitability for its intended use. See MPEP 2144.07.

Claims 44-50 and 53-57 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin et al. (US Patent 6,110,491) in view of Koch et al. (US Patent 5,538,954) as applied to claims 13, 16-19, 21, 22, 25-28, 30-32, 34-36, 39-42, 94, 95, 97, 98, 100, 101,156-158, 161,162, and 164 above, and further in view of Rahman (WO 03/030864 A1), as evidenced by CAS Registry Record for 23214-92-8 (doxorubicin), entered STN 16 Nov 1984 and as evidenced by CAS Registry Record for 97682-44-5 (irinotecan), entered STN 18 August 1985.

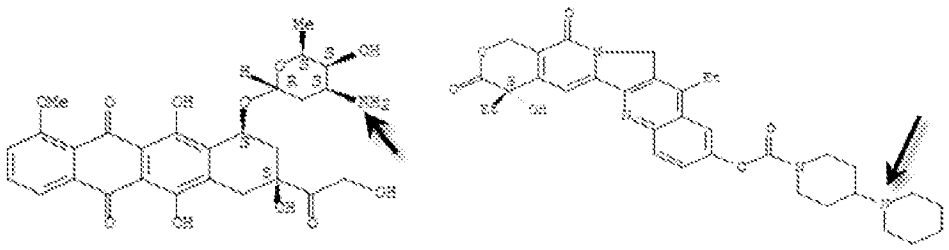
Kirpotin in view of Koch teaches a liposome comprising an active agent and a polymer with the opposite charge, wherein said polymer may be sucrose octasulfate. See the above rejection. Kirpotin teaches the use of doxorubicin as the active agent,

which is a known anti-cancer agent ionizable to a positive charge, as of Kirpotin, column 12 Example 1.

The references above do not teach irinotecan.

Rahman et al. (WO 03/030864 A1) (hereafter referred to as Rahman) teaches a liposomal composition comprising the active agent irinotecan which is used for treating cancer, as of the abstract of Rahman. Rahman teaches liposome encapsulated irinotecan, as of page 5 lines 3-6 and other places. Rahman, page 1 lines 5-10 teaches that irinotecan treats cancer. Rahman also teaches, as of page 7 lines 1-8, that irinotecan reduces cancer cells to develop resistance to other agents like doxorubicin.

It would have been prima facie obvious for one of ordinary skill in the art to have combined irinotecan for doxorubicin, as both irinotecan and doxorubicin are anti-cancer agents, and both compounds with be ionizable to form a positive charge because they contain amine functionalities. This would be understood by the skilled artisan from the structures of doxorubicin (reproduced from CAS Registry Record for 23214-92-8 below on the left) and irinotecan (reproduced from CAS Registry Record for 97682-44-5 below on the right) with arrows pointing to the protonatable nitrogen.



As both of the above structures contain nitrogen atoms that are protonatable, the skilled artisan would have been able to have predictably loaded both doxorubicin and

irinotecan into the liposome of the above references as both positively charged compounds would have predictably precipitated with anionic sucrose octasulfate with a reasonable expectation of success. Generally, it is *prima facie* obvious to combine two compositions, each of which is taught by the prior art to be useful for same purpose, in order to form a third composition to be used for the very same purpose. The idea for combining them flows logically from their having been individually taught in the prior art. See MPEP 2144.06.

Claims 96, 99, 102-113, and 119 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin et al. (US Patent 6,110,491) in view of Koch et al. (US Patent 5,538,954) as applied to claims 13, 16-19, 21, 22, 25-28, 30-32, 34-36, 39-42, 94, 95, 97, 98, 100, 101,156-158, 161,162, and 164 above, and further in view of Ahmad et al. (Cancer Research, Vol. 53, April 1993, pp. 1484-1488).

Kirpotin in view of Koch teaches a liposome comprising an active agent and a polymer with the opposite charge, wherein said polymer may be sucrose octasulfate. See the above rejection. Kirpotin teaches the use of PEG derivatized DSPE, as of column 9 lines 58-67, wherein said lipids comprise approximately 1/16 of the total lipids by mole.

The above references do not teach a specific molecular weight of PEG. The above references do not teach a targeting moiety.

Ahmad et al. (hereafter referred to as Ahmad) teaches liposomes comprising doxorubicin, lipid containing derivatives of polyethylene glycol, and a targeting moiety

wherein said targeting moiety is comprised of antibodies, as of Ahmad, page 1484, left column, abstract. The liposomes were prepared with PEG of a molecular weight of 19(: Daltons, (see Ahmad, page 1484, left column, third footnote, bottom of page), and wer prepared with a molar ratio of HSPC:cholesterol:DSPE-PEG (wherein HSPC is phosphatidylcholine and DSPE-PEG is the lipid distearoylethanolamine that is bonded to PEG) that is 2:1:0.1, as of Ahmad, page 1484, right column, section entitled "Liposome Preparation." Said formulation results in a mole fraction of 1/31 (3.2%) of DSPE-PEG. Ahmad teaches that liposomes with PEG but in the absence of antibody results in approximately 37.5% of liposomes remaining in the blood after 24 hours, as compared to undetectable amounts of liposomes remaining after 24 hours for those liposomes that lack PEG, as of Ahmad, page 1485, right column, last paragraph. Ahmad teaches animals injected with doxorubicin encapsulated into pegylated liposomes with an antibody experienced a greater reduction in lung tumor size as compared to animals injected with liposomes a targeting moiety, and that mice injected with liposomes comprising a targeting moiety survived longer than mice injected with liposomes lacking a targeting moiety, as of Ahmad, page 1487, left column, Table 1 and Figure 3.

It would have been prima facie obvious for one of ordinary skill in the art to have utilized PEG-1900 and a targeting moiety with the liposome made by the combination of Kirpotin. This is because the presence of PEG-1900 would have predictably in a longer half life of liposomal doxorubicin, as of Ahmad, page 1485, right column, last paragraph, resulting in greater tumor therapy with a reasonable expectation of success. The skilled

artisan would have further been motivated to have added an antibody targeting moiety because such a moiety predictably leads to significant tumor reduction and increase in survival rates with a reasonable expectation of success, as of as of Ahmad, page 1487, left column, Table 1 and Figure 3.

Claims 105-111, 113-116, and 119 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin et al. (US Patent 6,110,491) in view of Koch et al. (US Patent 5,538,954) and Ahmad et al. (Cancer Research, Vol. 53, April 1993, pp. 1484-1488) as applied to claims 13, 16-19, 21, 22, 25-28, 30-32, 34-36, 39-42, 94, 95, 97, 98, 100, 101,156-158, 161, 162, and 164 and claims 96, 99, 102-113, and 119 above, and further in view of Hong et al. (Annals of the New York Academy of Sciences, Vol. 886, 1999, pp. 293-296).

Kirpotin in view of Koch teaches a liposome comprising an active agent and a polymer with the opposite charge, wherein said polymer may be sucrose octasulfate. Ahmad teaches a targeting moiety. See the above rejection. Ahmad teaches animals injected with doxorubicin encapsulated into pegylated liposomes with an antibody experienced a greater reduction in lung tumor size as compared to animals injected with liposomes a targeting moiety, and that mice injected with liposomes comprising a targeting moiety survived longer than mice injected with liposomes lacking a targeting moiety, indicating that the targeting moiety has an anti-cancer effect, as of Ahmad, page 1487, left column, Table 1 and Figure 3.

The references above do not teach a moiety, wherein said moiety targets an ErbB2 (HER-2) receptor.

Hong et al. (hereafter referred to as Hong), teaches that HER-2 is highly overexpressed in cancers, especially breast cancer, as of page 293, second paragraph. Hong further teaches that the presence of an anti-HER2 targeting moiety in a doxorubicin immunoliposome (e.g. pegylated liposome) produced "marked antitumor effects," as of Hong, page 293, last paragraph.

It would have been prima facie obvious for one of ordinary skill in the art to have combined the pegylated, doxorubicin comprising liposomes of in view of Kirpotin with an ErbB2 targeting moiety, as said moiety would have predictably yielded an improvement in cancer targeting with a reasonable expectation of success, as of Hong, page 293, last paragraph. The formulations of Kirpotin and Hong use liposomal doxorubicin for the purposes of treating cancer, and the antibody of Hong would have predictably improved the cancer targeting of the liposome of Kirpotin with a reasonable expectation of success. Generally, it is *prima facie* obvious to combine two compositions, each of which is taught by the prior art to be useful for same purpose, in order to form a third composition to be used for the very same purpose. The idea for combining them flows logically from their having been individually taught in the prior art. See MPEP 2144.06.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ISAAC SHOMER whose telephone number is (571)270-7671. The examiner can normally be reached on 8:00 AM - 5:00 PM Monday-Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Frederick F. Krass can be reached on (571)272-0580. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/I. S./
Examiner, Art Unit 1612

/Frederick Krass/
Supervisory Patent Examiner, Art Unit 1612

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/121,294	05/02/2005	Keelung Hong	HERM1130-1	2502

28213 7590 12/06/2010

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EXAMINER	
SHOMER, ISAAC	

ART UNIT	PAPER NUMBER
1612	

MAIL DATE	DELIVERY MODE
12/06/2010	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	11/121,294	HONG ET AL.	
	Examiner	Art Unit	
	ISAAC SHOMER	1612	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *04 November 2010*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *See Continuation Sheet* is/are pending in the application.

4a) Of the above claim(s) *117,118,120-125,132-135,138-148,153-155 and 159* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *13,16-19,25,26,34-36,39-42,44-49,53-57,94-116,119 and 165* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *9 August 2010*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Continuation of Disposition of Claims: Claims pending in the application are 13,16-19,25,26,34-36,39-42,44-49,53-57,94-125,132-135, 138-148,153-155,159 and 165.

DETAILED ACTION

Applicants' arguments, filed 4 November 2010, have been fully considered. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn. The following rejections and/or objections are either reiterated or newly applied. They constitute the complete set presently being applied to the instant application.

Claim Rejections - 35 USC § 112 1st Paragraph: New Matter

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 13, 16-19, 25, 26, 34-36, 39-42, 46-49, 55-57, 94-116, and 119 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Applicant's amendment to claim 13 requiring a cationic anti-neoplastic entity is new matter not supported in the specification or original claims. Nowhere in the specification does applicant disclose the genus of cationic anti-neoplastic entities; while cationic agents are disclosed (page 26 paragraph 0094), and

anti-neoplastic agents are disclosed (e.g. page 23 paragraph 0086), nowhere is the entire genus of cationic anti-neoplastic agents disclosed.

Claim Rejections - 35 USC § 103

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 13, 16-19, 25, 26, 34-36, 39-42, 94, 95, 97, 98, 100, and 101 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin et al. (US Patent 6,110,491) in view of Schlessinger et al. (US Patent 5,783,568).

Kirpotin et al. (hereafter referred to as Kirpotin) teaches a liposome composition comprising an encapsulated [pharmaceutically active] compound in a stable precipitated form, wherein the concentration of said active compound is higher in the interior space of the liposome than in the medium surrounding the liposome, as of Kirpotin, abstract. The interior space of the liposome of Kirpotin includes an ionizable active compound and a charged precipitating agent (as of Kirpotin, column 2 lines 40-43) where the precipitating agent has a charge opposite to that of the ionizable active compound, as of Kirpotin, column 3 lines 24-27. In one example, the precipitating agent is a charged polymer, wherein said polymer has a molecular weight of 400 to 2 million daltons, as of Kirpotin, column 4 line 61 to column 5 line 8. Kirpotin suggests a compound that ionizes to a positive charge, and a precipitating agent that is a multivalent acid such as a polysulfate, as of Kirpotin, column 4 lines 20-29. In a more specific example, the active compound is doxorubicin which is cationic and the precipitating agent is an anion of

phosphate, sulfate, citrate, polyacrylate, or other anions, as of Kirpotin, column 11 lines 55-58. Kirpotin also teaches the use of a pH or electrochemical ion gradient, wherein said gradient has the same charge as the compound to be loaded, as of Kirpotin, column 3 lines 23-31. Ammonium ion gradients are suggested as of Kirpotin, Example 6, column 13 line 64. Parenteral administration is suggested as of Kirpotin, column 8 lines 39-40. In one example, Kirpotin teaches a doxorubicin ratio of 8 nmol/micromole for liposomes loaded without a polymeric sugar (as of Kirpotin Example 1 (column 12 lines 5-36)) and in a loading ratio of 26 nmol/micromole with the use of the anionic polymeric precipitating agent chondroitin sulfate (as of Kirpotin Example 3, (column 12 line 62 to column 13 lines 1-6, which also refers back to Example 1)). Said liposomes with precipitating agent comprise 5 mg/mL (which is equivalent to grams per liter) of chondroitin sulfate in the inner buffer (i.e. liposome interior), as of Kirpotin, column 12 lines 63-67. Kirpotin teaches the encapsulation of doxorubicin (an anti-cancer agent) into a liposome at 129 nanomole per micromole, which is a ratio of at least about 0.10 moles of therapeutic entity to moles of lipid as of Kirpotin, column 12 Example 1. Kirpotin teaches the use of PEG derivatized DSPE (pegylated DSPE), as of column 9 lines 58-67, wherein said pegylated lipids comprise approximately 1/16 of the total lipids by mole.

Kirpotin does not teach sucrose octasulfate (the elected specie of precipitating agent).

Schlessinger et al. (hereafter referred to as Schlessinger) teaches a salt or complex of a sulfated saccharide for use in treating cancer, as of Schlessinger, abstract.

In one embodiment, the compound used for anti-cancer purposes is sucrose octasulfate, as of Schlessinger, column 27 line 24, wherein sucrose octasulfate is taught to prevent oligomerization of heparin growth factor. Said composition may be administered parenterally, and liposomes are suggested as a delivery vehicle for parenteral administration, as of Schlessinger, column 12 lines 32-38.

It would have been prima facie obvious for one of ordinary skill in the art to have combined sucrose octasulfate, as of Schlessinger, with the liposome of Kirpotin. This is because the liposome of Kirpotin is used to carry known anti-cancer agents such as doxorubicin, as of Kirpotin, column 12 Example 1. As sucrose octasulfate is also an anti-cancer agent, as taught by Schlessinger, the skilled artisan would have been motivated to have combined sucrose octasulfate with the liposome of Kirpotin to have predictably treated cancer with a reasonable expectation of success. Generally, it is *prima facie* obvious to combine two compositions, each of which is taught by the prior art to be useful for same purpose, in order to form a third composition to be used for the very same purpose. The idea for combining them flows logically from their having been individually taught in the prior art. See MPEP 2144.06.

While the above references do not teach a polyol (such as inositol hexaphosphate), this is not required by the claims, as claim 13 is drawn to a "polyol or a sugar" as of claim 13, fourth line in claim, indicating that the presence of either a polyanionized polyol or a polyanionized sugar would meet the claim.

Claims 44-49, 53-57, and 165 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin et al. (US Patent 6,110,491) in view of Schlessinger et al. (US Patent 5,783,568) as applied to claims 13, 16-19, 25, 26, 34-36, 39-42, 94, 95, 97, 98, 100, and 101 above, and further in view of Rahman (WO 03/030864 A1).

Kirpotin in view of Schlessinger teach a liposomal composition comprising doxorubicin and sucrose octasulfate for treating cancer. See the above rejection.

The above references do not teach irinotecan.

Rahman et al. (WO 03/030864 A1) (hereafter referred to as Rahman) teaches a liposomal composition comprising the active agent irinotecan which is used for treating cancer, as of the abstract of Rahman. Rahman teaches liposome encapsulated irinotecan, as of page 5 lines 3-6 and other places. Rahman, page 1 lines 5-10 teaches that irinotecan treats cancer. Rahman also teaches, as of page 7 lines 1-8, that irinotecan reduces cancer cells to develop resistance to other agents like doxorubicin.

It would have been prima facie obvious for one of ordinary skill in the art to have combined irinotecan with the liposome of the above references (which already comprises doxorubicin and sucrose octasulfate). As irinotecan, doxorubicin, and sucrose octasulfate are all anti-cancer agents, the skilled artisan would have been motivated to have combined all of these components in a liposome to have predictably treated cancer with a reasonable expectation of success. Generally, it is *prima facie* obvious to combine two compositions, each of which is taught by the prior art to be useful for same purpose, in order to form a third composition to be used for the very

same purpose. The idea for combining them flows logically from their having been individually taught in the prior art. See MPEP 2144.06.

Claims 96, 99, 102-113 and 119 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin et al. (US Patent 6,110,491) in view of Schlessinger et al. (US Patent 5,783,568) as applied to claims 13, 16-19, 25, 26, 34-36, 39-42, 94, 95, 97, 98, 100, and 101 above, and further in view of Ahmad et al. (Cancer Research, Vol. 53, April 1993, pp. 1484-1488).

Kirpotin in view of Schlessinger teach a liposomal composition comprising doxorubicin and sucrose octasulfate for treating cancer. See the above rejection. Kirpotin teaches the use of PEG derivatized DSPE, as of column 9 lines 58-67, wherein said lipids comprise approximately 1/16 of the total lipids by mole.

The above references do not teach a specific molecular weight of PEG. The above references do not teach a targeting moiety.

Ahmad et al. (hereafter referred to as Ahmad) teaches liposomes comprising doxorubicin, lipid containing derivatives of polyethylene glycol, and a targeting moiety wherein said targeting moiety is comprised of antibodies, as of Ahmad, page 1484, left column, abstract. The liposomes were prepared with PEG of a molecular weight of 19(: Daltons, (see Ahmad, page 1484, left column, third footnote, bottom of page), and wer prepared with a molar ratio of HSPC:cholesterol:DSPE-PEG (wherein HSPC is phosphatidylcholine and DSPE-PEG is the lipid distearoylethanolamine that is bonded to PEG) that is 2:1:0.1, as of Ahmad, page 1484, right column, section entitled

"Liposome Preparation." Said formulation results in a mole fraction of 1/31 (3.2%) of DSPE-PEG. Ahmad teaches that liposomes with PEG but in the absence of antibody results in approximately 37.5% of liposomes remaining in the blood after 24 hours, as compared to undetectable amounts of liposomes remaining after 24 hours for those liposomes that lack PEG, as of Ahmad, page 1485, right column, last paragraph. Ahmad teaches animals injected with doxorubicin encapsulated into pegylated liposomes with an antibody experienced a greater reduction in lung tumor size as compared to animals injected with liposomes a targeting moiety, and that mice injected with liposomes comprising a targeting moiety survived longer than mice injected with liposomes lacking a targeting moiety, as of Ahmad, page 1487, left column, Table 1 and Figure 3.

It would have been prima facie obvious for one of ordinary skill in the art to have utilized PEG-1900 and a targeting moiety with the liposome made by the combination of Kirpotin. This is because the presence of PEG-1900 would have predictably in a longer half life of liposomal doxorubicin, as of Ahmad, page 1485, right column, last paragraph, resulting in greater tumor therapy with a reasonable expectation of success. The skilled artisan would have further been motivated to have added an antibody targeting moiety because such a moiety predictably leads to significant tumor reduction and increase in survival rates with a reasonable expectation of success, as of as of Ahmad, page 1487, left column, Table 1 and Figure 3.

Claims 105-111, 113-116, and 119 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin et al. (US Patent 6,110,491) in view of Schlessinger et al. (US Patent 5,783,568) and Ahmad et al. (Cancer Research, Vol. 53, April 1993, pp. 1484-1488) as applied to claims 13, 16-19, 25, 26, 34-36, 39-42, 94, 95, 97, 98, 100, and 101 and claims 96, 99, 102-113, and 119 above, and further in view of Hong et al. (Annals of the New York Academy of Sciences, Vol. 886, 1999, pp. 293-296).

Kirpotin in view of Schlessinger teach a liposomal composition comprising doxorubicin, irinotecan, and sucrose octasulfate. See the above rejection. Ahmad teaches a targeting moiety. See the above rejection. Ahmad teaches animals injected with doxorubicin encapsulated into pegylated liposomes with an antibody experienced a greater reduction in lung tumor size as compared to animals injected with liposomes a targeting moiety, and that mice injected with liposomes comprising a targeting moiety survived longer than mice injected with liposomes lacking a targeting moiety, indicating that the targeting moiety has an anti-cancer effect, as of Ahmad, page 1487, left column, Table 1 and Figure 3.

The above references do not teach a moiety, wherein said moiety targets an ErbB2 (HER-2) receptor.

Hong et al. (hereafter referred to as Hong), teaches that HER-2 is highly overexpressed in cancers, especially breast cancer, as of page 293, second paragraph. Hong further teaches that the presence of an anti-HER2 targeting moiety in a doxorubicin immunoliposome (e.g. pegylated liposome) produced "marked antitumor effects," as of Hong, page 293, last paragraph.

It would have been prima facie obvious for one of ordinary skill in the art to have combined the pegylated, doxorubicin comprising liposomes of in view of Kirpotin with an ErbB2 targeting moiety, as said moiety would have predictably yielded an improvement in cancer targeting with a reasonable expectation of success, as of Hong, page 293, last paragraph. The formulations of Kirpotin and Hong use liposomal doxorubicin for the purposes of treating cancer, and the antibody of Hong would have predictably improved the cancer targeting of the liposome of Kirpotin with a reasonable expectation of success.

Allegations of Unexpected Results

In applicant's arguments dated 4 November 2010 (hereafter referred to as applicant's arguments), applicant contends that the claimed invention has properties that are not suggested by the teachings of the prior art. Specifically, the prior art of Kirpotin is drawn to loading the anti-cancer drug doxorubicin into a liposome. The largest amount of doxorubicin loaded by Kirpotin into a liposome was 129 nmol of doxorubicin per micromole of liposomal phospholipid, as of Kirpotin, column 12 Example 1 (lines 33-34). In contrast, the present invention is drawn to irinotecan loaded into liposomes comprising inositol hexaphosphate (which is not present in Kirpotin), as of pages 116-117, Examples 66 and 67 of the specification, and table 36, which is reproduced below.

Drug	Input drug/lipid ratio, g/mol phospholipid	Encapsulated drug/lipid ratio, g/mol phospholipid	Loading efficiency, %
Vinorelbine	175	175.3 ± 8.0	100.2 ± 4.5
Vinorelbine	350	352.3 ± 11.8	100.6 ± 3.3
CPT-11	250	265.1 ± 11.2	106.1 ± 4.7
CPT-11	500	518.7 ± 27.8	103.7 ± 5.8

In the examples above, applicant has loaded CPT-11 (irinotecan) into liposomes comprising inositol hexaphosphate at amounts up to 518.7 grams irinotecan per mole phospholipid, which is equivalent to about 883 nanomole irinotecan per micromole of phospholipid, as per applicant's calculation on the footnote of page 18.

Applicant also points to an embodiment comprising 579.3 micrograms iriontecan per micromole phospholipid, which is calculated as being equivalent to 987.42 nanomoles irinotecan per micromole phospholipid. This is on pages 62-63, Example 13 and Table 9, reproduced below.

Extrusion membrane pore size, nm	Liposome size, nm mean SD	Drug load, mg Irinotecan/mmol phospholipid	Drug remaining in the liposomes after 24 hours in mice, % of pre-injection value
50	87.6±28.1	579.3± 24.2	79.2±3.8
80	98.5±15.1	571.1 ± 69.7	82.6 ± 2.1
100	110.8±25.2	567.7 ± 37.7	86.2 ± 2.7

In this liposome (the preparation of which is described in Example 11), applicant uses liposomes comprising sucrose octasulfate (abbreviated as SOS) as a trimethylammonium salt, as of the specification, page 57, paragraph 0160.

In response, it is the examiner's position that applicant's results would have been expected based upon the teachings of Kirpotin. Kirpotin is drawn to a liposome comprising an ionizable compound combined with a precipitating agent, wherein said precipitating agent causes concentrations that are severalfold higher than the bulk-

phase medium, as of Kirpotin, page 2 lines 53-57. Kirpotin suggests that said loading compound may be a non-polymeric, multivalent, ionically charged compound, as of Kirpotin, column 11 lines 15-22. As both sucrose octasulfate and inositol hexaphosphate are ionic and multivalent, the skilled artisan would have expected that these compounds would have predictably acted as precipitating agents with a reasonable expectation of success.

Even if, purely *en arguendo*, the magnitude of the increased loading were unexpected, the rejection would still be considered proper as the claims are not commensurate in scope with the evidence presented by applicant. See MPEP 716.02(d). Example 11 of applicant's specification requires a liposome comprising distearoyl phosphatidylcholine (DSPC), cholesterol, and distearoyl phosphatidylethanolamine conjugated to polyethylene glycol (DSPE-PEG), as of applicant's arguments, page 56, paragraph 0159, none of which are required by independent claim 13, as well as the drug irinotecan, and the triethylammonium salt of sucrose octasulfate, as page 58 of the specification. These ingredients are not required together by a single claim, and there is no evidence that the results presented herein would be applicable to any liposome with an interior space comprising any polyanionized sugar, any lipid composition, or any cationic anti-cancer agent. In examples 66 and 67 of the specification, applicant describes the preparation of liposomes comprising triethylammonium inositol hexaphosphate, the lipids DSPC, cholesterol, and PEG-DSPE (as of page 116 paragraph 0280 of the specification) and comprising the drugs vinorelbine or CPT-11, as of page 117 of the specification. None

of these ingredients are found together in one claim, and there is no evidence that the results presented here would be applicable to any liposome with an interior space comprising any polyanionized polyol, any lipid composition, or any cationic anti-cancer agent. Furthermore, the drug to lipid ratios obtained by applicant's experimentation are not required by independent claim 13.

Conclusion

No claim is allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

Applicant's submission of an information disclosure statement under 37 CFR 1.97(c) with the fee set forth in 37 CFR 1.17(p) on 9 August 2010 prompted the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 609.04(b). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any

extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ISAAC SHOMER whose telephone number is (571)270-7671. The examiner can normally be reached on 8:00 AM - 5:00 PM Monday-Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Frederick F. Krass can be reached on (571)272-0580. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ISAAC SHOMER/
Examiner, Art Unit 1612

/Frederick Krass/
Supervisory Patent Examiner, Art Unit 1612

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/121,294	05/02/2005	Keelung Hong	HERM1130-1	2502

28213 7590 04/13/2011

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EXAMINER	
SHOMER, ISAAC	
ART UNIT	**PAPER NUMBER**
1612	

MAIL DATE	DELIVERY MODE
04/13/2011	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	11/121,294	HONG ET AL.	
	Examiner	Art Unit	
	ISAAC SHOMER	1612	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *07 March 2011*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *See Continuation Sheet* is/are pending in the application.

4a) Of the above claim(s) *117,118,120-125,132-135,138-148,153-155 and 159* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *13,16-19,25,26,34-36,39-42,44-49,53-57,94-116,119 and 165* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *8 December 2010*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Continuation of Disposition of Claims: Claims pending in the application are 13,16-19,25,26,34-36,39-42,44-49,53-57,94-125,132-135,138-148,153-155,159 and 165.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114.

Applicant's submission filed on 7 March 2011 has been entered, and the arguments presented therein have been fully considered. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn. The following rejections and/or objections are either reiterated or newly applied. They constitute the complete set presently being applied to the instant application.

Claim Rejections - 35 USC § 103 – Upheld Rejections

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 13, 16-19, 25, 26, 34-36, 39-42, 94, 95, 97, 98, 100, and 101 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin et al. (US Patent 6,110,491) in view of Schlessinger et al. (US Patent 5,783,568).

A) In applicant's arguments dated 7 March 2011 (hereafter referred to as applicant's arguments), applicant contends that the combination of any two therapeutic

entities is non-obvious because the ability to assemble functional combination compositions in the pharmaceutical arts is completely unpredictable, as of applicant's arguments, page 14, bottom paragraph. In fact, cancer-treating compositions in which two agents that are known to treat cancer individually are combined to form a combination therapy are taught as of Miles et al. (The Oncologist, 2002, 7(suppl 6), pages 13-19) (hereafter referred to as Miles). Miles teaches breast cancer therapies which include combinations of paclitaxel and trastuzumab (as of Miles, page 13, abstract), capecitabine and docetaxel (as of Miles, page 13, abstract), anthracyclines with taxanes (as of Miles, page 15, Table 1), anthracyclines with cyclophosphamides and fluorouracil (as of Miles, page 15, Table 1), and doxorubicin with vinorelbine (as of Miles, page 15, Table 2).

Furthermore, the Applicant has provided not evidence to support the assertion of the predictability or unpredictability of the prior art or the expectations of success of one of ordinary skill in the art at the time the invention was made. Rather, the statement has been put forth without any supporting evidence.

B) Applicant argues that the combination of two pharmaceutical agents is non-obvious, in contrast to the detergent arts, which was alleged to have been cited by the previous office action (dated 6 December 2010).

In response, the combination of two or more chemical agents known from many chemical arts for the same purpose is prima facie obvious. See the discussion of the combination two or more chemical agents for anti-cancer purposes above.

Furthermore, the combination of two known herbicides for the same purpose is prima facie obvious. See Ex parte Quadranti, 25 USPQ2d 1071 (Bd. Pat. App. & Inter. 1992), as of MPEP 2144.06(I), last sentence. As herbicides are understood to be pharmaceutical agents, the MPEP does teach that the combination of two pharmaceutical agents for the same purpose is prima facie obvious.

C) Applicant argues that "it is a matter of fundamental chemistry of salt formations that when two compounds [a cationic compound an anionic compound] are entrapped together, they are forced to be present at fixed stoichiometry (i.e. a fixed molar ratio), as of applicant's arguments, page 15, top paragraph. As such, applicant is arguing that independent claim 13 specifies a "fixed molar ratio" of cationic anti-neoplastic agent to polyanionized polyol in view of the fact that the claim recites that the formulation must be in the form of an acid or a salt.

The examiner agrees that in a salt, the number of cationic sites must balance the number of anionic sites for the salt to remain electrically neutral. However, the word "salt" does not imply a "fixed stoichiometry." For example, a solid composition comprising an equimolar ratio of sodium and chloride is a salt, and is known as table salt. However, a solid composition in which 100% of the cations are sodium, 50% of the anions are chloride, and another 50% of the anions are bromide is also a salt. Therefore, the term "salt" may imply a fixed stoichiometry of total cationic species to total anionic species, but does not require specific ratios among the different cationic species and different anionic species present in the composition.

The claims utilize the transitional phrase “comprising,” indicating that other entities may be present besides those specifically recited in the claim. See MPEP 2111.03, second paragraph, regarding the open-ended interpretation of the term "comprising."

Therefore, contrary to applicant’s assertions on page 15, top paragraph of applicant’s arguments, the claims do not require that the cationic anti-neoplastic therapeutic agent and the polyanionized sugar or polyol be present at a fixed molar ratio.

D) Applicant argues that different drugs have different pharmacokinetic and pharmacodynamic properties, citing a textbook edited by Moore, pages 10-13 (attached with applicant’s previous communication). Applicant argues that it is better to increase the frequency of the dosing rather than the amount of drug given, and the adjustments of doses typically require different dosing schedules. As such, it appears applicant is arguing that the skilled artisan would not use a combination therapy because it would result in an increase of the amount of drug given.

This argument is not persuasive. While different anti-cancer drugs may be combined, such a combination does not directly imply that the total dosage of the drug has been increased. The examiner considers a hypothetical dosage consisting of 100 mg of paclitaxel, and a second hypothetical dosage form consisting of 100 mg docetaxel. A combination dosage of 50 mg of paclitaxel and 50 mg of docetaxel would contain two different drugs, but would not result in the delivery of more drug individually. Therefore, applicant's arguments against combination therapies is not persuasive.

E) Applicant contends that the claimed liposomal composition provides a fixed ratio of two entities within the liposome, as of applicant's arguments, paragraph bridging pages 15 and 16.

The examiner disagrees. There is no fixed ratio of any component recited in independent claim 13. Furthermore, there is no fixed ratio of cationic anti-neoplastic agent to polyanionized sugar/polyol recited in claim 13 or any other pending claim. As such, applicant is arguing limitations that are not claimed. Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See MPEP 2145(VI).

Claims 44-49, 53-57, and 165 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin et al. (US Patent 6,110,491) in view of Schlessinger et al. (US Patent 5,783,568) as applied to claims 13, 16-19, 25, 26, 34-36, 39-42, 94, 95, 97, 98, 100, and 101 above, and further in view of Rahman (WO 03/030864 A1).

Claims 96, 99, 102-113 and 119 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin et al. (US Patent 6,110,491) in view of Schlessinger et al. (US Patent 5,783,568) as applied to claims 13, 16-19, 25, 26, 34-36, 39-42, 94, 95, 97, 98, 100, and 101 above, and further in view of Ahmad et al. (Cancer Research, Vol. 53, April 1993, pp. 1484-1488).

Claims 105-111, 113-116, and 119 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin et al. (US Patent 6,110,491) in view of Schlessinger et al. (US Patent 5,783,568) and Ahmad et al. (Cancer Research, Vol. 53, April 1993, pp.

1484-1488) as applied to claims 13, 16-19, 25, 26, 34-36, 39-42, 94, 95, 97, 98, 100, and 101 and claims 96, 99, 102-113, and 119 above, and further in view of Hong et al. (Annals of the New York Academy of Sciences, Vol. 886, 1999, pp. 293-296).

In applicant's arguments, pages 16-19, applicant contends that the dependent claims are non-obvious for the same reason that the independent claim is non-obvious. No further arguments regarding the subject matter of the dependent claims is presented.

This is not persuasive. Independent claim 13 is obvious for the reasons described above. As no further arguments are presented with respect to the dependent claims, the rejections are maintained.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ISAAC SHOMER whose telephone number is (571)270-7671. The examiner can normally be reached on 8:00 AM - 5:00 PM Monday-Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Frederick F. Krass can be reached on (571)272-0580. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/ISAAC SHOMER/
Examiner, Art Unit 1612

/Frederick Krass/
Supervisory Patent Examiner, Art Unit 1612

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/121,294	05/02/2005	Keelung Hong	HERM1130-1	2502

28213 7590 07/12/2011

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EXAMINER	
SHOMER, ISAAC	

ART UNIT	PAPER NUMBER
1612	

MAIL DATE	DELIVERY MODE
07/12/2011	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Interview Summary	Application No.	Applicant(s)
	11/121,294	HONG ET AL.
	Examiner	Art Unit
	ISAAC SHOMER	1612

All participants (applicant, applicant's representative, PTO personnel):

(1) *ISAAC SHOMER (Examiner)*.	(3) *Seth A. Fidel (Reg. 38,449)*.
(2) *Patricia Duffy (Examiner)*.	(4) *Edward Robinson (Reg. 43,049)*.

Date of Interview: *06 July 2011*.

Type: a)☒ Telephonic b)☐ Video Conference
c)☒ Personal [copy given to: 1)☐ applicant 2)☒ applicant's representative]

Exhibit shown or demonstration conducted: d)☒ Yes e)☐ No.
If Yes, brief description: *Applicant presented various scientific journal articles used to show unexpected results*.

Claim(s) discussed: *13,16-19,25,26,34-36,39-42,44-49,53-57,94-116,119 and 165*.

Identification of prior art discussed: *Kirpotin et al. (US Patent 6,110,491), Schlessinger et al. (US Patent 5,783,568)*.

Agreement with respect to the claims f)☐ was reached. g)☒ was not reached. h)☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: *Data was presented that was used in an attempt to show that the inventive subject matter has unexpected and superior results. Further claim limitations were discussed that would be commensurate in scope with the results. Applicants will submit results as an amendment. It was also noted that examiner Brian Gulledge was present. It is further noted that attorney Seth Fidel was present in person, whereas attoreney Edward Robinson participated telephonically*.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN A NON-EXTENDABLE PERIOD OF THE LONGER OF ONE MONTH OR THIRTY DAYS FROM THIS INTERVIEW DATE, OR THE MAILING DATE OF THIS INTERVIEW SUMMARY FORM, WHICHEVER IS LATER, TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached sheet.

/Patricia A Duffy/ Primary Examiner, Art Unit 1645		

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/121,294	05/02/2005	Keelung Hong	HERM1130-1	2502

28213 7590 11/23/2011

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EXAMINER	
SHOMER, ISAAC	

ART UNIT	PAPER NUMBER
1612	

MAIL DATE	DELIVERY MODE
11/23/2011	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	11/121,294	HONG ET AL.	
	Examiner	Art Unit	
	ISAAC SHOMER	1612	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 and 16 August 2011*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *166-181,183 and 184* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *166-181,183 and 184* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *16 August 2011, 20 October 2011*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicants' arguments, filed 12 August and 16 August 2011, have been fully considered. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn. The following rejections and/or objections are either reiterated or newly applied. They constitute the complete set presently being applied to the instant application.

The examiner clarifies that the claims filed in the supplemental response dated 16 August 2011 are under examination.

Claim Rejections - 35 USC § 103

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 166, 167, 170-173, 176, 181, and 183 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chou et al. (Journal of Bioscience and Bioengineering, Vol. 95, No. 4, 2003, pages 405-408) in view of Schlessinger et al. (US Patent 5,783,568).

Chou et al. (hereafter referred to as Chou) is drawn to liposomal irinotecan prepared by a pH gradient loading method, as of Chou, page 405, abstract. Said liposomes include neutral lipids such as phosphatidylcholine and uncharged lipids such as cholesterol, as of Chou, page 405, right column, first full paragraph. Irinotecan is useful for the treatment of cancer, as of Chou, page 405 left column, first paragraph.

Chou suggests the inclusion of sulfated oligosaccharides, as of Chou, page 408 left column, middle of first full paragraph.

Chou does not teach sucrose octasulfate or inositol hexaphosphate.

Schlessinger et al. (hereafter referred to as Schlessinger) teaches a salt or complex of a sulfated saccharide for use in treating cancer, as of Schlessinger, abstract. In one embodiment, the compound used for anti-cancer purposes is sucrose octasulfate, as of Schlessinger, column 27 line 24, wherein sucrose octasulfate is taught to prevent oligomerization of heparin growth factor. Said composition may be administered parenterally, and liposomes are suggested as a delivery vehicle for parenteral administration, as of Schlessinger, column 12 lines 32-38, and as such would have been present in a medium and would not have been solid.

It would have been prima facie obvious for one of ordinary skill in the art to have combined sucrose octasulfate, as of Schlessinger, with the liposome comprising irinotecan of Chou. This is because the liposome of Chou comprises an irinotecan, as of Chou, page 405. As sucrose octasulfate is also an anti-cancer agent, as taught by Schlessinger, the skilled artisan would have been motivated to have combined sucrose octasulfate with the liposome of Chou to have predictably treated cancer with a reasonable expectation of success. Generally, it is prima facie obvious to combine two compositions, each of which is taught by the prior art to be useful for same purpose, in order to form a third composition to be used for the very same purpose. The idea for combining them flows logically from their having been individually taught in the prior art. See MPEP 2144.06.

As to claims 170-173, the property of being active at reducing human colon carcinoma would have been expected by the compositions of the prior art because irinotecan is a known anti-cancer agent. As to the comparisons between liposomal irinotecan and free irinotecan, it is noted that Chou teaches liposomal irinotecan.

As to claim 176, the skilled artisan would have expected that the liposome of Chou would have had the stability required by the claim at a temperature of 2-8 C. This is because Chou teaches lipids such as cholesterol, as of Chou, page 405 right column, first full paragraph, which are known to be used for liposome stability.

As to claim 181, Chou teaches the inclusion of DSPE-PEG in the liposome, as of page 406 right column, second full paragraph, wherein PEG is polyethylene glycol.

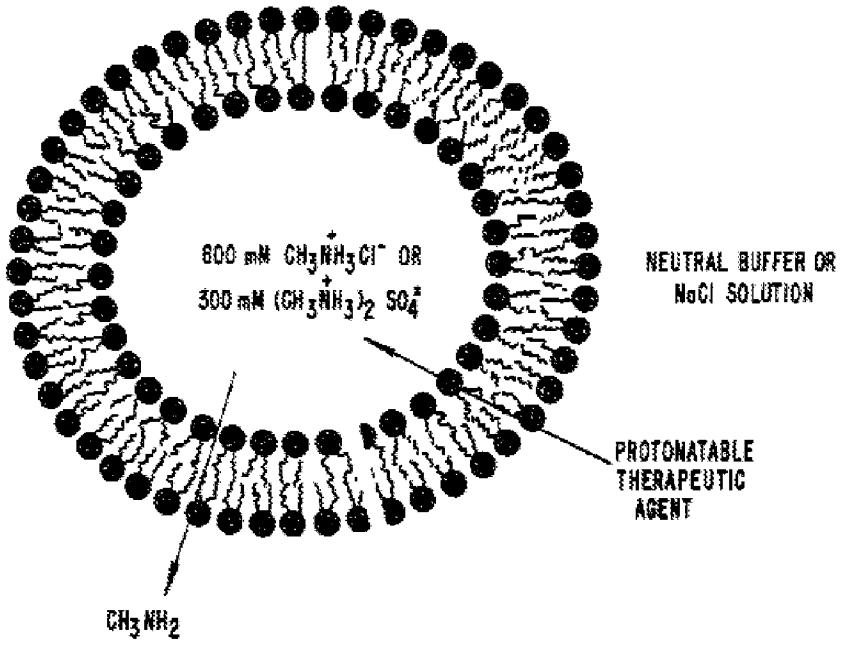
As to claim 183, Chou does not explicitly teach parenteral administration, as all experiments done by Chou appear to have been done in vitro. However, the skilled artisan would have realized that liposomal compositions are applicable for in vivo parenteral administration.

Claim 178 is rejected under 35 U.S.C. 103(a) as being unpatentable over Chou et al. (Journal of Bioscience and Bioengineering, Vol. 95, No. 4, 2003, pages 405-408) in view of Schlessinger et al. (US Patent 5,783,568) as applied to claims 166, 167, 170-173, 176, 181, and 183 above, and further in view of Hope et al. (US Patent 5,785,987).

Chou teaches a liposome comprising irinotecan. Chou in view of Schlessinger teach a liposome comprising irinotecan and sucrose octasulfate. See the above rejection over of Chou and Schlessinger by themselves.

Neither Chou nor Schlessinger teach an entrapped ammonium compound.

Hope et al. (hereafter referred to as Hope) is drawn to the use of a methylamine gradient for loading a therapeutic agent across the lipid barrier of a liposome, wherein said therapeutic agent is protonatable, as of Hope, abstract and Figure 1, reproduced below.



The use of methylamine to load a protonatable drug results in rapid loading and retention, as of Hope, column 10 lines 54-56, and appears to be successful regardless of the pH of the composition, as of Hope, column 11 lines 53-56.

It would have been prima facie obvious for one of ordinary skill in the art to have used the methylamine gradient of Hope to have loaded irinotecan into a liposome, as of Chou. This is because the methylamine gradient of Hope is useful for loading protonatable therapeutic agents into liposomes rapidly and with good retention, as taught by Hope. As irinotecan is a protonatable therapeutic agent (it comprises a tertiary

amine nitrogen bonded to three sp3 hybridized carbon atoms), the skilled artisan would have been motivated to have used methylamine to have predictably loaded and retained irinotecan into the liposome of Chou with a reasonable expectation of success.

Claims 179 and 180 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chou et al. (Journal of Bioscience and Bioengineering, Vol. 95, No. 4, 2003, pages 405-408) in view of Schlessinger et al. (US Patent 5,783,568) and Hope et al. (US Patent 5,785,987) as applied to claims 166, 167, 170-173 176, 178, 181, and 183 above, and further in view of Katsu et al. (Anal. Chem., Vol. 73, 2001, pages 1849-1854).

Chou teaches a liposome comprising irinotecan. Chou in view of Schlessinger teach a liposome comprising irinotecan and sucrose octasulfate. Hope teaches a methylammonium ion gradient. See the above rejection over of Chou, Schlessinger, and Hope by themselves.

Chou, Schlessinger, and Hope do not teach a triethylammonium ion gradient.

Katsu et al. (hereafter referred to as Katsu) teaches a triethylamine and triethylammonium ion gradient as a pH gradient, as of Katsu, page 1850 left column, equation (2) and first full paragraph. Said gradient is across a liposome, as of Katsu, page 1852, right column, first full paragraph.

It would have been prima facie obvious for one of ordinary skill in the art to have used a triethylammonium ion gradient, as of Katsu, for the ammonium ion gradient in the liposomes of the above references. This is because triethylammonium ion would

have been useful for gradient for loading liposomes. As such, one of ordinary skill in the art would have been motivated to have substituted triethylammonium ions for methylammonium ions to have predictably created an ion gradient into a liposome with a reasonable expectation of success. The simple substitution of one known element (triethylammonium ion gradient, as of Katsu) for another (methylammonium ion gradient, as of Hope) to obtain predictable results (loading of irinotecan into a liposome) is prima facie obvious. See MPEP 2143, Exemplary Rationale B.

Claims 166, 167, 170-173, and 183 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rahman et al. (WO 2003/030864 A1) in view of Schlessinger et al. (US Patent 5,783,568).

Rahman et al. (hereafter referred to as Rahman) teaches a liposomal composition comprising the active agent irinotecan which is used for treating cancer, as of the abstract of Rahman. Rahman teaches liposome encapsulated irinotecan, as of page 5 lines 3-6 and other places. Rahman, page 1 lines 5-10 teaches that irinotecan treats cancer. Rahman also teaches, as of page 7 lines 1-8, that irinotecan reduces cancer cells to develop resistance to other agents like doxorubicin. Said liposomes comprise lipids such as phosphatidylcholine, which is neutral, and cholesterol, which is not charged, as of Rahman, page 2 lines 15-18.

Rahman does not teach sucrose octasulfate or inositol hexaphosphate.

Schlessinger et al. (hereafter referred to as Schlessinger) teaches a salt or complex of a sulfated saccharide for use in treating cancer, as of Schlessinger, abstract.

In one embodiment, the compound used for anti-cancer purposes is sucrose octasulfate, as of Schlessinger, column 27 line 24, wherein sucrose octasulfate is taught to prevent oligomerization of heparin growth factor. Said composition may be administered parenterally, and liposomes are suggested as a delivery vehicle for parenteral administration, as of Schlessinger, column 12 lines 32-38, and as such would have been present in a medium and would not have been solid.

It would have been prima facie obvious for one of ordinary skill in the art to have combined sucrose octasulfate, as of Schlessinger, with the liposome comprising irinotecan of Rahman. This is because the liposome of Rahman comprises an irinotecan, which treats cancer, as the abstract of Rahman. As sucrose octasulfate is also an anti-cancer agent, as taught by Schlessinger, the skilled artisan would have been motivated to have combined sucrose octasulfate with the liposome of Rahman to have predictably treated cancer with a reasonable expectation of success. Generally, it is prima facie obvious to combine two compositions, each of which is taught by the prior art to be useful for same purpose, in order to form a third composition to be used for the very same purpose. The idea for combining them flows logically from their having been individually taught in the prior art. See MPEP 2144.06.

As to claims 170-173, the property of being active at reducing human colon carcinoma would have been expected by the compositions of the prior art because irinotecan is a known anti-cancer agent. As to the comparisons between liposomal irinotecan and free irinotecan, it is noted that Rahman teaches liposomal irinotecan.

As to claim 183, Rahman teaches parenteral administration, as of page 6 line 21.

Claims 166, 167, 170-173, 181, 183, and 184 are rejected under 35 U.S.C. 103(a) as being unpatentable over Slater et al. (US 2002/0146450 A1) in view of Schlessinger et al. (US Patent 5,783,568).

Slater et al. (hereafter referred to as Slater) is drawn to a liposomal composition comprising a topoisomerase inhibitor, as of Slater, abstract. Said topoisomerase inhibitor may be irinotecan, as of Slater, paragraph 0017.

Slater does not teach sucrose octasulfate or inositol hexaphosphate.

Schlessinger et al. (hereafter referred to as Schlessinger) teaches a salt or complex of a sulfated saccharide for use in treating cancer, as of Schlessinger, abstract. In one embodiment, the compound used for anti-cancer purposes is sucrose octasulfate, as of Schlessinger, column 27 line 24, wherein sucrose octasulfate is taught to prevent oligomerization of heparin growth factor. Said composition may be administered parenterally, and liposomes are suggested as a delivery vehicle for parenteral administration, as of Schlessinger, column 12 lines 32-38, and as such would have been present in a medium and would not have been solid.

It would have been prima facie obvious for one of ordinary skill in the art to have combined sucrose octasulfate, as of Schlessinger, with the liposome comprising irinotecan as of Slater. This is because irinotecan, being a topoisomerase inhibitor, is known for treating cancer, as of Slater, paragraph 0005. As sucrose octasulfate also has anti-cancer effects, as taught by Schlessinger, the skilled artisan would have been motivated to have combined sucrose octasulfate as of Schlessinger with liposomal

irinotecan to have predictably made a composition that treats cancer with a reasonable expectation of success. Generally, it is *prima facie* obvious to combine two compositions, each of which is taught by the prior art to be useful for same purpose, in order to form a third composition to be used for the very same purpose, in this case the purpose being cancer treatment. The idea for combining them flows logically from their having been individually taught in the prior art. See MPEP 2144.06.

As to claims 170-173, the property of being active at reducing human colon carcinoma would have been expected by the compositions of the prior art because irinotecan is a known anti-cancer agent. As to the comparisons between liposomal irinotecan and free irinotecan, it is noted that Slater teaches liposomal irinotecan.

As to claim 181, Slater teaches PEG-DSPE, as of paragraph 0095.

As to claim 183, Slater teaches that the dose is injected, as of paragraph 0099, wherein injectable administration is a form of parenteral administration.

As to claim 184, Slater teaches an amount of preferably at least about 0.20 micromole drug per micromole lipid. This overlaps with the 1:1 drug:lipid molar ratio required by claim 184. While the prior art does not disclose the exact claimed values, but does overlap: in such instances even a slight overlap in range establishes a *prima facie* case of obviousness. In re Peterson, 65 USPQ2d 1379, 1382 (Fed. Cir. 2003).

Claims 166, 168, 170-175, 181, 183, and 184 are rejected under 35 U.S.C. 103(a) as being unpatentable over Slater et al. (US 2002/0146450 A1) in view of

(HighBeam Research, "From Antinutrient to Phytonutrient: Phytic Acid Gains Respect" Environmental Nutrition, 1 April 2004).

Slater et al. (hereafter referred to as Slater) is drawn to a liposomal composition comprising a topoisomerase inhibitor, as of Slater, abstract. Said topoisomerase inhibitor may be irinotecan, as of Slater, paragraph 0017.

Slater does not teach sucrose octasulfate or inositol hexaphosphate.

HighBeam Research (hereafter referred to as HighBeam) teaches that inositol hexaphosphate (also known as phytic acid) has a tumor-blocking effect in laboratory experiments, as of Slater, first page, fourth full paragraph.

It would have been prima facie obvious for one of ordinary skill in the art to have combined inositol hexaphosphate, as of HighBeam, with the liposome comprising irinotecan as of Slater. This is because irinotecan, being a topoisomerase inhibitor, is known for treating cancer, as of Slater, paragraph 0005. As inositol hexaphosphate also has anti-cancer effects, as taught by HighBeam, the skilled artisan would have been motivated to have combined inositol hexaphosphate with liposomal irinotecan to have predictably made a composition that treats cancer with a reasonable expectation of success. Generally, it is *prima facie* obvious to combine two compositions, each of which is taught by the prior art to be useful for same purpose, in order to form a third composition to be used for the very same purpose, in this case the purpose being cancer treatment. The idea for combining them flows logically from their having been individually taught in the prior art. See MPEP 2144.06.

As to claims 170-173, the property of being active at reducing human colon carcinoma would have been expected by the compositions of the prior art because irinotecan is a known anti-cancer agent. As to the comparisons between liposomal irinotecan and free irinotecan, it is noted that Slater teaches liposomal irinotecan.

As to claim 174, HighBeam teaches inositol hexaphosphate, which is required by this claim.

As to claim 181, Slater teaches PEG-DSPE, as of paragraph 0095.

As to claim 183, Slater teaches that the dose is injected, as of paragraph 0099, wherein injectable administration is a form of parenteral administration.

As to claim 184, Slater teaches an amount of preferably at least about 0.20 micromole drug per micromole lipid. This overlaps with the 1:1 drug:lipid molar ratio required by claim 184. While the prior art does not disclose the exact claimed values, but does overlap: in such instances even a slight overlap in range establishes a *prima facie* case of obviousness. In re Peterson, 65 USPQ2d 1379, 1382 (Fed. Cir. 2003).

As to claim 175, Slater teaches a molar ratio of at least 0.2:1 drug to lipid, as explained in the above paragraph. Given a molecular weight of irinotecan of about 587 grams per mole, this is a ratio of at least 117 grams of drug per mole of lipid. This overlaps with the claimed ratio of 500 grams of drug per mole of lipid.

Response to Applicant's Arguments Regarding Allegations of Unexpected Results

Part 1: Response to Arguments Pertaining to Chou et al. and Slater et al.: Liposome Comprising Irinotecan and Sucrose Octasulfate:

Applicant presented arguments specifically regarding Chou on page 13 and pages 16-17 of applicant's arguments dated 12 August 2011. Applicant cites Figures 1, 3, and 4 of Chou, which are alleged to indicate that none of Chou's liposomal irinotecan retained as much as 50% of the drug over 4 hours (e.g. has a half-life of four hours). In contrast, applicant cites page 63, Example 14 of the specification, which includes both irinotecan and sucrose octasulfate, which is alleged to show a drug-retention half-life of 56.8 hours. This example cites Figure 5 of the instant drawings. Figure 1 of Chou and Figure 5 of the instant disclosure and shown below, side-by-side, for comparison. The prior art figure is on the left and the instant figure on the right.

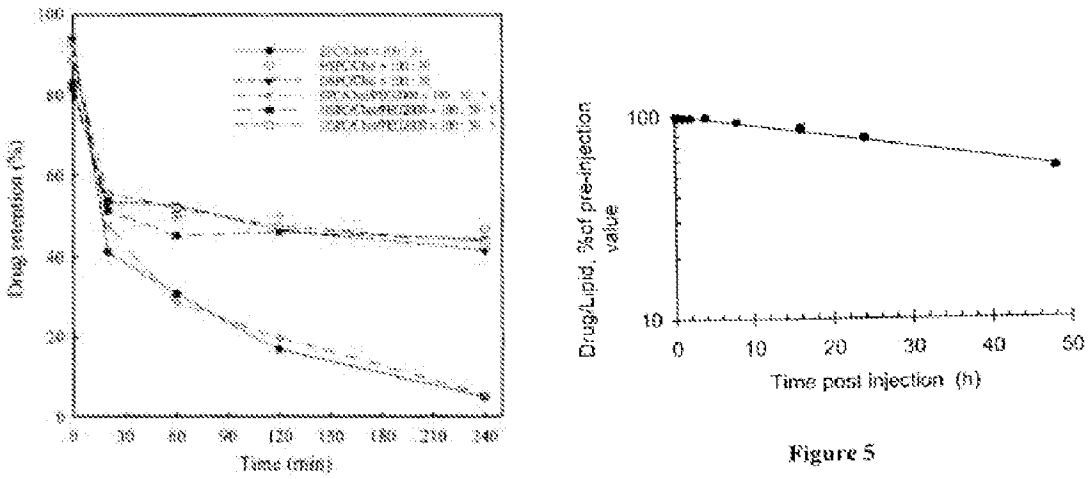


Figure 5

The examiner agrees that the specification shows an unexpected increase in drug and liposomal retention as compared with Chou.

Nevertheless, the results presented by applicant are not commensurate in scope with independent instant claim 166 for the following reasons:

A) Use of the term “medium”

The claims require that the liposomes be present in a medium. The term "medium” as currently recited is understood as reading on both a lipidic medium and an aqueous medium. However, if the medium were lipidic, there would be no separation between the lipid bilayer of the liposome and the medium. Furthermore, the drug release kinetics would be different in a lipidic medium than an aqueous medium.

Applicant’s use of the term “medium” is not commensurate in scope with applicant’s data, wherein the data is only drawn to an aqueous medium. As such, applicant is encouraged to replace the term “medium” with "aqueous medium” so that the claims can be commensurate in scope with applicant’s showing.

B) Half-Release Time.

Applicant’s data, as of Figure 5, appears to show a half-release time of greater than 48 hours. In contrast, Slater teaches a half-release time of about 14 hours, as of Slater, paragraph 0099. As such, applicant’s data is not commensurate in scope with claim 166, which does not limit the half-release time. However, applicant's data is commensurate in scope with the requirement of claim 168 (which requires a half-release time of greater than 24 hours) in the case where the liposome includes irinotecan and sucrose octasulfate. This is because applicant’s data teaches a liposome comprising irinotecan and sucrose octasulfate with a half-release time that unexpectedly and significantly exceeds that of the prior art.

Part 2: Response to Allegations of Unexpected Results Regarding Ko and Drummond References:

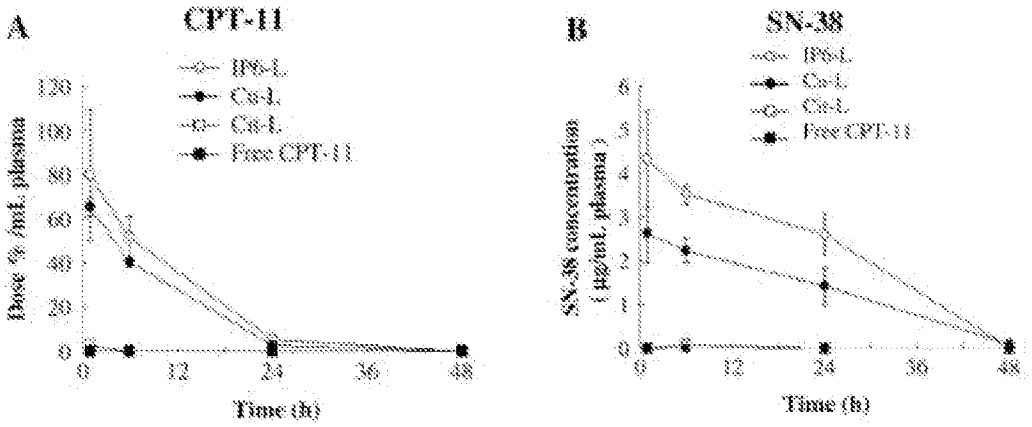
Ko et al. (poster presentation) (hereafter referred to as Ko). This reference compares the claimed liposomal irinotecan formulation to non-liposomal gemcitabine. See Ko, "Introduction" section, box bridging first and second columns of poster. The formulation “PEP02” is the claimed liposomal irinotecan formulation.

Applicant also cited the reference Drummond et al. (Cancer Research, Vol. 66(6), 2006, pages 3271-3277). This reference compares liposomes comprising irinotecan (abbreviated CPT-11) and sucrose octasulfate or inositol hexaphosphate to free irinotecan. See Drummond, page 3274, Table 1.

To rebut the prima facie case of obviousness, the claimed invention must be compared with the closest prior art. See MPEP 716.02(e). In this case, the comparative formulation of Ko is non-liposomal gemcitabine, and the comparative formulation in Drummond is non-liposomal irinotecan. In contrast, the closest prior art is Rahman, Chou, and Slater which both teach liposomal irinotecan in the absence of sucrose octasulfate or inositol hexaphosphate. As such, the prior art formulations of Rahman, Chou, and Slater are closer to the claimed invention than the comparative formulation used by Ko or Drummond. Therefore, neither Ko nor Drummond are probative of non-obviousness.

Part 3: Response to Arguments Pertaining to Hattori et al: Liposome Comprising Irinotecan and Inositol Hexaphosphate:

Hattori et al. (Journal of Controlled Release, Vol. 136, 2009, pages 30-37) (hereafter referred to as Hattori). Hattori compares a liposome comprising irinotecan and inositol hexaphosphate with a different liposome which comprises irinotecan but lacks inositol hexaphosphate (irinotecan is abbreviated as CPT-11 in Hattori). The liposome of the invention is referred to as IP6-L, and the comparative liposome lacking inositol hexaphosphate is referred to as Cit-L. Hattori shows that the inventive liposome, (which includes IP6) results in irinotecan and SN-38 being present in the bloodstream for a significantly longer period of time than what is the case for the comparative liposome. This is shown by Hattori, page 32 left column, Figures 2A and 2B, reproduced below side-by-side.



In contrast, Slater teaches the following half release data, as of Slater, Figures 1A and 4A, reproduced below.

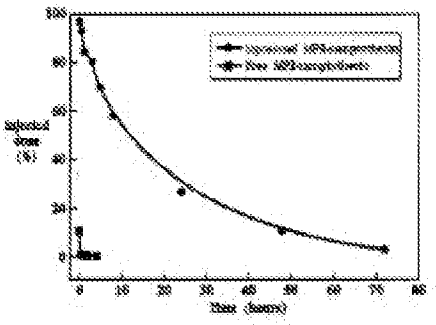


Fig. 1A

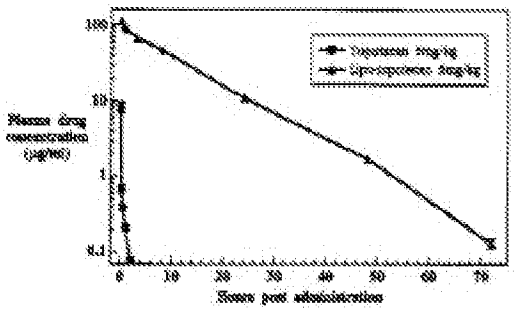


Fig. 4A

The half-release times presented by applicant in Hattori do not appear to be significantly improved as compared with the half-release of Slater. As such, applicant has not shown an unexpectedly improved half-release time regarding the combination of liposomal irinotecan with inositol hexaphosphate.

Conclusion

No claim is allowed at this time.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any

extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ISAAC SHOMER whose telephone number is (571)270-7671. The examiner can normally be reached on 8:00 AM - 5:00 PM Monday-Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Frederick F. Krass can be reached on (571)272-0580. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ISAAC SHOMER/
Examiner, Art Unit 1612

/Frederick Krass/
Supervisory Patent Examiner, Art Unit 1612

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/601,451	11/17/2006	Daryl C. Drummond	HERM1130-2	5211

28213 7590 01/11/2010

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EXAMINER
KISHORE, GOLLAMUDI S

ART UNIT	PAPER NUMBER
1612	

MAIL DATE	DELIVERY MODE
01/11/2010	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	11/601,451	DRUMMOND ET AL.	
	Examiner	Art Unit	
	GOLLAMUDI S. KISHORE	1612	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-40* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-40* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *6-4-07; 3-11-09; 10-23-09*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Claims included in the prosecution are 1-40.

Claim Rejections - 35 USC § 112

1. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

2. Claims 2-4 and 10 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

'topoisomerase I inhibitor' in claim 2 lacks an antecedent basis in claim 1.

The examiner suggests reciting the expanded form of MRT in claim 3.

'hours' is misspelled on line 3 of claim 4.

It is unclear as to what applicant intends to convey by 'substituted ammonium ion gradient' and 'solubility gradient' in claim 10.

Claim Rejections - 35 USC § 103

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

4. Claims 1-5, 15-21 and 36 are rejected under 35 U.S.C. 103(a) as being unpatentable over Anderson (US 2002/0049176).

Anderson while disclosing modulation of mitochondrial mass and function for the treatment of diseases teaches that for the delivery into the brain the compositions may be injected into the brain via cannula (0397). The therapeutic agents include camptothecin (0160) and the delivery devices taught by Anderson are liposomes (0273, 0315, 0322, 0350, 0357, 0393, and 0394). It would have been obvious to one of ordinary skill in the art to delver camptothecins directly to the brain using a cannula with a reasonable expectation of success since Anderson is suggestive of the use of camptothecins and liposomes as the delivery vehicles. Since liposomes are art known sustained release vehicles, it would have been obvious to one of ordinary skill in the art that the residence times of the liposomes of WO would be greater than the residence times of SN-38 which is injected directly in a non-encapsulated form.

5. Claims 22-30, 33-34 and 37-40 are rejected under 35 U.S.C. 103(a) as being unpatentable over Anderson (US 2002/0049176) in combination with Torchilin (5,534,241).

The teachings of Anderson have been discussed above. What is lacking in Anderson's liposomes is the presence of a detectable marker such as gadolinium (GD).

Torchilin discloses liposomal compositions containing chelating groups and labeling ions such as Gadolinium for imaging a target region of the body of the patient. The liposomes further contain a targeting group such as an antibody.(Abstract, col. 5,

lines 20-24; col. 7, lines 1-54 col. 9, line 60 through col. 10, line 31; col. 12, lines 31-67 and claims).

It would have been obvious to one of ordinary skill in the art to use detectable markers in the liposomes of Slater with a reasonable expectation of success since Torchilin teaches that liposomes can be attached to ions such as Gd through chelating moieties to image a target region of the body of the patient. Although Torchilin does not teach the claimed chelator in claim 30, he teaches several chelating agents on col. 5, lines 5-19 and further teaches that a variety of chelating agents could be used depending on the desired labeling ion. Therefore, it would have been obvious to one of ordinary skill in the art to use any chelating agent including the claimed compound with the expectation of obtaining similar results. Supplying the composition in a kit form would have been obvious to one of ordinary skill in the art since it is routinely practiced in medical art.

6. Claims 1-5, 15-21 and 36 are rejected under 35 U.S.C. 103(a) as being unpatentable over WO 2004/017940 in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819).

WO 2004 teaches liposomes containing SN 38 (camptothecin derivative) for the treatment of brain cancers. The liposomes containing phospholipids and PEG-derivatized phospholipids. The liposome can be made by using ion gradients (abstract, page 3, line 28 through page 4, line 19; pages 5-7; page 12, line 23; examples). Although WO does not disclose other topoisomerase inhibitors, it would have been

obvious to one of ordinary skill in the art to use other topoisomerase inhibitors with a reasonable expectation of success especially in view of Slater teaches the effectiveness of various topoisomerase inhibitors in liposomes against cancers and the method of preparation.

What are lacking in WO are the teachings of the administration of SN-38 directly into the brain using a catheter or needle

Anderson while disclosing modulation of mitochondrial mass and function for the treatment of diseases teaches that for the delivery into the brain the compositions may be injected into the brain via cannula (0397). The therapeutic agents include camptothecin (0160).

Kaplitt teaches that delivery devices such as liposomes can be injected directly into the brain using a catheter (0003 and claim 1).

Gillis similarly teaches chemotherapeutic agents in liposomes can be delivered to a tumor in the brain of the mammal using a catheter (0032, claims 1 and 7).

Gourrdie teaches liposomes containing active agents can be directly delivered into the brain via catheter or needle (0067, 0074, 0084-0088, 0105, 0109-0110).

It would have been obvious to one of ordinary skill in the art to inject the liposomal compositions of WO directly into the brain with a reasonable expectation of success since Anderson teaches that compositions containing camptothecin can be directly injected into the brain via cannula. Since liposomes are art known sustained release vehicles, it would have been obvious to one of ordinary skill in the art that the residence times of the liposomes of WO would be greater than the residence times of

SN-38 which is injected directly in a non-encapsulated form. One of ordinary skill in the art would be motivated to inject the compositions of WO directly into the brain since liposomes can be injected directly into the brain using a catheter as taught by Kaplitt or Gourrdie or Gillies who teaches the delivery of liposomes containing chemotherapeutic agents into the brain using a catheter.

7. Claims 22-30, 33-34 and 37-40 are rejected under 35 U.S.C. 103(a) as being unpatentable over WO 2004/017940 in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819) as set forth above, further in view of Torchilin (5,534,241).

The teachings of WO, Anderson, Kaplitt, Gillis and Gourrdie have been discussed above. What is lacking in WO and Anderson's liposomes is the presence of a detectable marker such as gadolinium (GD).

Torchilin discloses liposomal compositions containing chelating groups and labeling ions such as Gadolinium for imaging a target region of the body of the patient. The liposomes further contain a targeting group such as an antibody.(Abstract, col. 5, lines 20-24; col. 7, lines 1-54 col. 9, line 60 through col. 10, line 31; col. 12, lines 31-67 and claims).

It would have been obvious to one of ordinary skill in the art to use detectable markers in the liposomes of WO or Anderson with a reasonable expectation of success since Torchilin teaches that liposomes can be attached to ions such as Gd through chelating moieties to image a target region of the body of the patient. Although Torchilin does not teach the claimed chelator in claim 30, he teaches several chelating agents on

col. 5, lines 5-19 and further teaches that a variety of chelating agents could be used depending on the desired labeling ion. Therefore, it would have been obvious to one of ordinary skill in the art to use any chelating agent including the claimed compound with the expectation of obtaining similar results. Supplying the composition in a kit form would have been obvious to one of ordinary skill in the art since it is routinely practiced in medical art.

8. Claims 1-21, 31, 32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Anderson (US 2002/0049176) OR WO 2004/017940 in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819) as set forth above, further in view of Slater (6,355,268).

The teachings of Anderson, WO, Kaplitt, Gillis, and Gourrdie have been discussed above.

What is lacking in Anderson and WO is the teaching of the use of other camptothecins and the loading of the camptothecins using an ion gradient.

Slater discloses liposomal formulations containing topoisomerase inhibitors (water soluble camptothecin derivatives) for the treatment of cancers. The liposomes contain PEG and have an inside/outside gradient to retain the topoisomerase inhibitor within the liposomes. The topoisomerase inhibitors include topotecans. Slater also teaches loading using a polyanionic polymer such as dextran sulfate to trap or retain the drug within the liposomes (Abstract, col. 2, line 49 through col. 3, line 67; col. 5, line 37 through col. 6, line 17; col. 17, line 37 through col. 9, line 22 through col. 12, line 45 through col. 13, line 67; examples and claims 18-26). Slater further teaches that

topoisomerase inhibitors are class of cancer therapy drugs which inhibit the action of topoisomerase enzymes which play a role in the replication, repair, genetic recombination and transcription of DNA (col. 1, lines 42-58).

It would have been obvious to one of ordinary skill in the art to use any camptothecin including those taught by Slater for the treatment of brain tumors with a reasonable expectation of success since these compounds act by inhibiting DNA replication and transcription of DNA as taught by Slater. The use of claimed loading method would have been obvious to one of ordinary skill in the art since this method is routinely practiced to load water soluble camptothecins as taught by Slater.

9. Claims 1-21, 31-32, 36 are rejected under 35 U.S.C. 103(a) as being unpatentable over Slater (6,355,268) in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819).

The teachings of Anderson, Kaplitt, Gillis, and Gourrdie have been discussed above.

Slater discloses liposomal formulations containing topoisomerase inhibitors for the treatment of cancers. The liposomes contain PEG and have an inside/outside gradient to retain the topoisomerase inhibitor within the liposomes. The topoisomerase inhibitors include topotecans. Slater also teaches loading using a polyanionic polymer such as dextran sulfate to trap or retain the drug within the liposomes (Abstract, col. 2, line 49 through col. 3, line 67; col. 5, line 37 through col. 6, line 17; col. 17, line 37 through col. 9, line 22 through col. 12, line 45 through col. 13, line 67; examples and

claims 18-26). Slater further teaches that topoisomerase inhibitors are class of cancer therapy drugs which inhibit the action of topoisomerase enzymes which play a role in the replication, repair, genetic recombination and transcription of DNA (col. 1, lines 42-58).

What is lacking in Slater is the treatment of brain cancer or the administration of the topoisomerase inhibitors via a conduit. The use of the topoisomerase inhibitors for brain cancer however, would have been obvious to one of ordinary skill in the art since Slater teaches that these compounds are useful in the treatment of cancers and one would expect similar results even with breast cancer. It would have been obvious to one of ordinary skill in the art to inject the liposomal compositions of WO directly into the brain with a reasonable expectation of success since Anderson teaches that compositions containing camptothecin can be directly injected into the brain via cannula. Since liposomes are art known sustained release vehicles, it would have been obvious to one of ordinary skill in the art that the residence times of the liposomes of WO would be greater than the residence times of SN-38 which is injected directly in a non-encapsulated form. One of ordinary skill in the art would be motivated to inject the compositions of WO directly into the brain since liposomes can be injected directly into the brain using a catheter as taught by Kaplitt or Gourrdie or Gillies who teaches the delivery of liposomes containing chemotherapeutic agents into the brain using a catheter.

10. Claims 22-30, 33-34 and 37-40 are rejected under 35 U.S.C. 103(a) as being unpatentable over Slater (6,355,268) in combination with Anderson (US 2002/0049176)

or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819) as set forth above, further in view of Torchilin (5,534,241).

The teachings of Slater, Anderson, Kaplitt, Gillis, and Gourrdie have been discussed above. What is lacking in Slater's liposomes is the presence of a detectable marker such as gadolinium (GD).

Torchilin discloses liposomal compositions containing chelating groups and labeling ions such as Gadolinium for imaging a target region of the body of the patient. The liposomes further contain a targeting group such as an antibody.(Abstract, col. 5, lines 20-24; col. 7, lines 1-54 col. 9, line 60 through col. 10, line 31; col. 12, lines 31-67 and claims).

It would have been obvious to one of ordinary skill in the art to use detectable markers in the liposomes of Slater with a reasonable expectation of success since Torchilin teaches that liposomes can be attached to ions such as Gd through chelating moieties to image a target region of the body of the patient. Although Torchilin does not teach the claimed chelator in claim 30, he teaches several chelating agents on col. 5, lines 5-19 and further teaches that a variety of chelating agents could be used depending on the desired labeling ion. Therefore, it would have been obvious to one of ordinary skill in the art to use any chelating agent including the claimed compound with the expectation of obtaining similar results. Supplying the composition in a kit form would have been obvious to one of ordinary skill in the art since it is routinely practiced in medical art.

11. Claims 33-35 are rejected under 35 U.S.C. 103(a) as being unpatentable over 1) Anderson (US 2002/0049176) in view of Torchillin; 2) WO 2004/017940 in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819), further in view of Torchillin; 3) Slater (6,355,268) in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819), in view of Torchillin all as set forth above, further in view of Yotnda (US 2003/0220284).

The teachings of Slater, Anderson, WO, Kaplitt, Gillis, and Gourrdie have been discussed above.

What is lacking in Slater is the teaching of the attachment of a ligand such as EGF which binds to the receptor.

Yotnda teaches that ligands such as EGF which specifically bind to the receptors can be functionally incorporated into a liposome membrane to direct the liposomes to that specific cell population. Yotnda further teaches camptothecin (0125, 0181 and 0182).

Attachment of a targeting ligand such as EGF in the liposomes of Slater would have been obvious to one of ordinary skill in the art if the cancer cells express the receptors for EGF as taught by Yotnda.

12. Claims 37-40 are rejected under 35 U.S.C. 103(a) as being unpatentable over 1) Anderson (US 2002/0049176); 2) WO 2004/017940 in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819); 3) Slater (6,355,268) in combination with Anderson (US

2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819) all as set forth above, further in view of Sarris (US 2004/0071768).

The teachings of Slater, Anderson, WO, Kaplitt, Gillis, and Gourrdie have been discussed above.

These references do not specifically teach a kit for the compositions.

Sarris while disclosing liposomal compositions for cancer therapy teaches that the compositions can be supplied in a kit form (0084, 0118, 0138 and 0156).

Supplying the liposomal compositions in a kit form would have been obvious to one of ordinary skill in the art with a reasonable expectation of success since Sarris shows that liposomal compositions can be supplied in a kit form.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to GOLLAMUDI S. KISHORE whose telephone number is (571)272-0598. The examiner can normally be reached on 6:30 AM- 4 PM, alternate Friday off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Krass Frederick can be reached on (571) 272-0580. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Gollamudi S Kishore/
Primary Examiner, Art Unit 1612

GSK

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/601,451	11/17/2006	Daryl C. Drummond	HERM1130-2	5211

28213 7590 08/27/2010

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EXAMINER
KISHORE, GOLLAMUDI S

ART UNIT	PAPER NUMBER
1612	

MAIL DATE	DELIVERY MODE
08/27/2010	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	11/601,451	DRUMMOND ET AL.	
	Examiner	Art Unit	
	GOLLAMUDI S. KISHORE	1612	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

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Status

1)☒ Responsive to communication(s) filed on *09 July 2010*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-47* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-47* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *7-9-10*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

The amendment dated 7-9-10 is acknowledged.

Claims included in the prosecution are 1-47.

To reduce the issues, the 103 rejections involving Anderson are withdrawn.

Claim Rejections - 35 USC § 103

1. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

2. Claims 1-5, 15-21 and 36 are rejected under 35 U.S.C. 103(a) as being unpatentable over WO 2004/017940 in combination with Anderson (US 2002/0049176), or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819).

WO 2004 teaches liposomes containing SN 38 (camptothecin derivative) for the treatment of brain cancers. The liposomes containing phospholipids and PEG-derivatized phospholipids. The liposome can be made by using ion gradients (abstract, page 3, line 28 through page 4, line 19; pages 5-7; page 12, line 23; examples). Although WO does not disclose other topoisomerase inhibitors, it would have been obvious to one of ordinary skill in the art to use other topoisomerase inhibitors with a reasonable expectation of success especially in view of Slater teaches the effectiveness

of various topoisomerase inhibitors in liposomes against cancers and the method of preparation.

What are lacking in WO are the teachings of the administration of SN-38 directly into the brain using a catheter or needle

Anderson while disclosing modulation of mitochondrial mass and function for the treatment of diseases teaches that for the delivery into the brain the compositions may be injected into the brain via cannula (0397).

Kaplitt teaches that delivery devices such as liposomes can be injected directly into the brain using a catheter (0003 and claim 1).

Gillis similarly teaches chemotherapeutic agents in liposomes can be delivered to a tumor in the brain of the mammal using a catheter (0032, claims 1 and 7).

Gourrdie teaches liposomes containing active agents can be directly delivered into the brain via catheter or needle (0067, 0074, 0084-0088, 0105, 0109-0110).

It would have been obvious to one of ordinary skill in the art to inject the liposomal compositions of WO directly into the brain with a reasonable expectation of success since Anderson teaches that compositions containing camptothecin can be directly injected into the brain via cannula. Since liposomes are art known sustained release vehicles, it would have been obvious to one of ordinary skill in the art that the residence times of the liposomes of WO would be greater than the residence times of SN-38 which is injected directly in a non-encapsulated form. One of ordinary skill in the art would be motivated to inject the compositions of WO directly into the brain since liposomes can be injected directly into the brain using a catheter as taught by Kaplitt or

Gourrdie or Gillies who teaches the delivery of liposomes containing chemotherapeutic agents into the brain using a catheter.

Applicant's arguments have been fully considered, but are not found to be persuasive. Applicant argues that the claimed invention distinguishes over the cited references on several fronts : by claiming methods for increasing the mean residence time of a topoisomerase I inhibitor (e.g., a camptothecin compound) in the brain tissue of a mammal without increasing toxicity, by: (a) providing the topoisomerase I inhibitor" compound in liposomally encapsulated form and (b) administering the liposomally encapsulated topoisomerase I inhibitor via a conduit placed into the brain of the mammal, wherein, even though the mean residence time in the brain is increased, the toxicity of the topoisomerase I inhibitor is no greater than or is less than the toxicity of a topoisomerase I inhibitor in a non-encapsulated form, when similarly administered in the brain via a conduit placed into the brain of the mammal. According to applicant, WO does not teach any method of increasing the mean residence time of a camptothecin compound in the brain of a subject without increasing the toxicity as required by the instant claims. These arguments are not found to be persuasive since liposomes are known in the art as sustained release vehicles and therefore, the residence time of any therapeutic agent would naturally be longer than the therapeutic agent alone since they deliver the agents slowly. Secondly, liposomes are known to reduce the toxicity of therapeutic agents. References of Janoff (5,059,591), Mayer (5,795,589), Carlsson (6,022,561), Radhakrishnan (5,043,165) are cited of interest in this context. (See col. 4, lines 49-63; col. 8, line 60 through col. 9, line 34 of Janoff; col. 2, line 59 through col. 3,

line 27 of Mayer; col. 5, lines 30-37 of Carlsson; col. 4, lines 3-25 of Radhakrishnan). Therefore, what is claimed is to be expected and not unexpected in nature.

Applicant's arguments that Anderson does not cure the defects of WO are not persuasive since Anderson teaches the delivery into the brain the compositions may be injected into the brain via cannula. Applicant's arguments that Kaplitt, Gillis and Goudie do not teach or suggest any methods for increasing the mean residence time of camptothecins in the brain tissue are not persuasive since these references are combined for the method of administration of compounds into the brain tissue.

3. Claims 1-5, 15-21, 36 and 41-47 are rejected under 35 U.S.C. 103(a) as being unpatentable over WO 2004/017940 in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819), further in view of WO 03/030864 of record or vice versa; that is, WO 03 in combination with WO 04, Anderson, Kaplitt, Gillis or Gourrdie.

The teachings of WO, Anderson, Kaplitt, Gillis and Gourrdie have been discussed above.

WO teaches SN 38 and not Irinotecan.

WO 03 teaches liposomal formulations containing Irinotecan for the treatment of mammalian cancers (abstract and claims). WO 03 further teaches that the liposomal encapsulation results in reduced toxicity, ability to administer drug as a bolus or short infusion in a high concentration and increased therapeutic efficacy of the drug (page 2, lines 31-36). WO 03 in addition teaches that SN-38 is a metabolite of Irinotecan (page 1, lines 10-14.

The use of Irinotecan instead of SN-38 in WO would have been obvious to one of ordinary skill in the art with a reasonable expectation of success since WO 03 teaches Irinotecan is also a potent anti-cancer drug and it is converted into SN-38. Alternately, the use of WO 03 formulations for brain cancer and administer the composition via conduit would have been obvious to one of ordinary skill in the art since WO 03 teaches the use of SN-38 for brain cancer and the references of Anderson, Kaplitt, Gillis and Gourrdie teach direct administration of the compositions into the brain.

4. Claims 22-30, 33-34 and 37-40 are rejected under 35 U.S.C. 103(a) as being unpatentable over WO 2004/017940 in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie OR over WO 2004/017940 in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819), further in view of WO 03/030864 of record or vice versa; that is, WO 03 in combination with WO 04, Anderson, Kaplitt, Gillis or Gourrdie (US2008/0095819) both set forth above, further in view of Torchilin (5,534,241).

The teachings of WO, Anderson, Kaplitt, Gillis and Gourrdie have been discussed above. What is lacking in WO and Anderson's liposomes is the presence of a detectable marker such as gadolinium (GD).

Torchilin discloses liposomal compositions containing chelating groups and labeling ions such as Gadolinium for imaging a target region of the body of the patient. The liposomes further contain a targeting group such as an antibody.(Abstract, col. 5,

lines 20-24; col. 7, lines 1-54 col. 9, line 60 through col. 10, line 31; col. 12, lines 31-67 and claims).

It would have been obvious to one of ordinary skill in the art to use detectable markers in the liposomes of WO or Anderson with a reasonable expectation of success since Torchilin teaches that liposomes can be attached to ions such as Gd through chelating moieties to image a target region of the body of the patient. Although Torchilin does not teach the claimed chelator in claim 30, he teaches several chelating agents on col. 5, lines 5-19 and further teaches that a variety of chelating agents could be used depending on the desired labeling ion. Therefore, it would have been obvious to one of ordinary skill in the art to use any chelating agent including the claimed compound with the expectation of obtaining similar results. Supplying the composition in a kit form would have been obvious to one of ordinary skill in the art since it is routinely practiced in medical art.

Applicant's arguments have been fully considered, but are not persuasive. The examiner has already addressed applicant's arguments regarding WO, Anderson, Kaplitt, Gillis and Gourrdie. Applicant argues that Torchillin does not cure the defects of WO. This argument is not persuasive since Torchillin is combined for its teachings of the use of detectable markers. The function of a detectable marker would remain the same irrespective of the active agent or liposomal composition or tissue which has to be detected.

5. Claims 1-21, 31, 32, 41-47 are rejected under 35 U.S.C. 103(a) as being unpatentable over WO 2004/017940 in combination with Anderson (US 2002/0049176)

or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819) OR over WO 2004/017940 in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819), further in view of WO 03/030864 of record or vice versa; that is, WO 03 in combination with WO 04, Anderson, Kaplitt, Gillis or Gourrdie both set forth above, further in view of Slater (6,355,268).

The teachings of Anderson, WO, Kaplitt, Gillis, and Gourrdie have been discussed above.

What is lacking in Anderson and WO is the teaching of the use of other camptothecins and the loading of the camptothecins using an ion gradient.

Slater discloses liposomal formulations containing topoisomerase inhibitors (water soluble camptothecin derivatives) for the treatment of cancers. The liposomes contain PEG and have an inside/outside gradient to retain the topoisomerase inhibitor within the liposomes. The topoisomerase inhibitors include topotecans and Irinotecan. Slater also teaches loading using a polyanionic polymer such as dextran sulfate to trap or retain the drug within the liposomes (Abstract, col. 2, line 49 through col. 3, line 67; col. 5, line 37 through col. 6, line 17; col. 17, line 37 through col. 9, line 22 through col. 12, line 45 through col. 13, line 67; examples and claims 18-26). Slater further teaches that topoisomerase inhibitors are class of cancer therapy drugs which inhibit the action of topoisomerase enzymes which play a role in the replication, repair, genetic recombination and transcription of DNA (col. 1, lines 42-58).

It would have been obvious to one of ordinary skill in the art to use any camptothecin including those taught by Slater for the treatment of brain tumors with a reasonable expectation of success since these compounds act by inhibiting DNA replication and transcription of DNA as taught by Slater. The use of claimed loading method would have been obvious to one of ordinary skill in the art since this method is routinely practiced to load water soluble camptothecins as taught by Slater.

Applicant's arguments have been fully considered, but are not persuasive. The examiner has already addressed applicant's arguments regarding WO, Anderson, Kaplitt, Gillis and Gourrdie. Applicant argues that Slater does not cure the defects of WO. This argument is not persuasive since Slater is combined for its teachings of the loading method and also for the teachings of several topoisomerase inhibitors. Applicant points out to col. 13, lines 26-34 of Slater and argue that the liposome encapsulated topoisomerase inhibitor formulations however, show increased toxicity compared to free drug. This argument is not persuasive. Slater teaches several topoisomerase inhibitors and his examples include topoisomerase inhibitors such as Topotecan. Slater's statement appears to be specific to MPE-camptothecin and instant claims include this compound and applicant has not shown that this compound acts differently in instant liposomes as opposed to Slater's liposomes.

6. Claims 1-21, 31-32, 36 and 41-47 are rejected under 35 U.S.C. 103(a) as being unpatentable over Slater (6,355,268) in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819).

The teachings of Anderson, Kaplitt, Gillis, and Gourrdie have been discussed above.

Slater discloses liposomal formulations containing topoisomerase inhibitors for the treatment of cancers. The liposomes contain PEG and have an inside/outside gradient to retain the topoisomerase inhibitor within the liposomes. The topoisomerase inhibitors include Topotecan. Slater also teaches loading using a polyanionic polymer such as dextran sulfate to trap or retain the drug within the liposomes (Abstract, col. 2, line 49 through col. 3, line 67; col. 5, line 37 through col. 6, line 17; col. 17, line 37 through col. 9, line 22 through col. 12, line 45 through col. 13, line 67; examples and claims 18-26). Slater further teaches that topoisomerase inhibitors are class of cancer therapy drugs which inhibit the action of topoisomerase enzymes which play a role in the replication, repair, genetic recombination and transcription of DNA (col. 1, lines 42-58).

What is lacking in Slater is the treatment of brain cancer or the administration of the topoisomerase inhibitors via a conduit. The use of the topoisomerase inhibitors for brain cancer however, would have been obvious to one of ordinary skill in the art since Slater teaches that these compounds are useful in the treatment of cancers and one would expect similar results even with breast cancer. It would have been obvious to one of ordinary skill in the art to inject the liposomal compositions of WO directly into the brain with a reasonable expectation of success since Anderson teaches that compositions containing camptothecin can be directly injected into the brain via cannula. Since liposomes are art known sustained release vehicles, it would have been

obvious to one of ordinary skill in the art that the residence times of the liposomes of WO would be greater than the residence times of SN-38 which is injected directly in a non-encapsulated form. One of ordinary skill in the art would be motivated to inject the compositions of WO directly into the brain since liposomes can be injected directly into the brain using a catheter as taught by Kaplitt or Gourrdie or Gillies who teaches the delivery of liposomes containing chemotherapeutic agents into the brain using a catheter.

Applicant's arguments have been fully considered, but are not persuasive. The examiner has already addressed applicant's arguments regarding all the references.

7. Claims 22-30, 33-34 and 37-40 are rejected under 35 U.S.C. 103(a) as being unpatentable over Slater (6,355,268) in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819) as set forth above, further in view of Torchilin (5,534,241).

The teachings of Slater, Anderson, Kaplitt, Gillis, and Gourrdie have been discussed above. What is lacking in Slater's liposomes is the presence of a detectable marker such as gadolinium (GD).

Torchilin discloses liposomal compositions containing chelating groups and labeling ions such as Gadolinium for imaging a target region of the body of the patient. The liposomes further contain a targeting group such as an antibody.(Abstract, col. 5, lines 20-24; col. 7, lines 1-54 col. 9, line 60 through col. 10, line 31; col. 12, lines 31-67 and claims).

It would have been obvious to one of ordinary skill in the art to use detectable markers in the liposomes of Slater with a reasonable expectation of success since Torchilin teaches that liposomes can be attached to ions such as Gd through chelating moieties to image a target region of the body of the patient. Although Torchilin does not teach the claimed chelator in claim 30, he teaches several chelating agents on col. 5, lines 5-19 and further teaches that a variety of chelating agents could be used depending on the desired labeling ion. Therefore, it would have been obvious to one of ordinary skill in the art to use any chelating agent including the claimed compound with the expectation of obtaining similar results. Supplying the composition in a kit form would have been obvious to one of ordinary skill in the art since it is routinely practiced in medical art.

Applicant's arguments have been fully considered, but are not persuasive. The examiner has already addressed applicant's arguments regarding all the references.

8. Claims 33-35 are rejected under 35 U.S.C. 103(a) as being unpatentable over 1) WO 2004/017940 in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819), further in view of Torchillin; 3) over WO 2004/017940 in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819), further in view of WO 03/030864 of record or vice versa; that is, WO 03 in combination with WO 04, Anderson, Kaplitt, Gillis or Gourrdie.

4) Slater (6,355,268) in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819), in view of Torchillin all as set forth above, further in view of Yotnda (US 2003/0220284).

The teachings of Slater, Anderson, WO, Kaplitt, Gillis, and Gourrdie have been discussed above.

What is lacking in Slater is the teaching of the attachment of a ligand such as EGF which binds to the receptor.

Yotnda teaches that ligands such as EGF which specifically bind to the receptors can be functionally incorporated into a liposome membrane to direct the liposomes to that specific cell population. Yotnda further teaches camptothecin (0125, 0181 and 0182).

Attachment of a targeting ligand such as EGF in the liposomes of Slater would have been obvious to one of ordinary skill in the art if the cancer cells express the receptors for EGF as taught by Yotnda.

Applicant's arguments have been fully considered, but are not persuasive. The examiner has already addressed applicant's arguments regarding the primary references. Applicant's only argument is that Yotnda does not cure the deficiencies of the primary references. This argument is not persuasive since this reference is combined for its teachings of the attachment ligands such as EGF to direct the liposomes to specific cell populations and this function would be the same irrespective of the nature of the active agent encapsulated.

9. Claims 37-40 are rejected under 35 U.S.C. 103(a) as being unpatentable over 1) WO 2004/017940 in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819); 3) over WO 2004/017940 in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819), further in view of WO 03/030864 of record or vice versa; that is, WO 03 in combination with WO 04, Anderson, Kaplitt, Gillis or Gourrdie; 4) Slater (6,355,268) in combination with Anderson (US 2002/0049176) or Kaplitt (US 2006/0129126) or Gillis (Us 2004/0243101) or Gourrdie (US2008/0095819) all as set forth above, further in view of Sarris (US 2004/0071768).

The teachings of Slater, Anderson, WO, Kaplitt, Gillis, and Gourrdie have been discussed above.

These references do not specifically teach a kit for the compositions.

Sarris while disclosing liposomal compositions for cancer therapy teaches that the compositions can be supplied in a kit form (0084, 0118, 0138 and 0156).

Supplying the liposomal compositions in a kit form would have been obvious to one of ordinary skill in the art with a reasonable expectation of success since Sarris shows that liposomal compositions can be supplied in a kit form.

Applicant's arguments have been fully considered, but are not found to be persuasive. The examiner has already addressed applicant's arguments regarding the primary references. Applicant argues that Sarris does not teach or suggest any reason, which would lead one of ordinary skill in the art to modify the teachings of Anderson or

WO in combination with other references. This argument is not persuasive since Sarris is combined for its teachings of the supply of the liposomal compositions in a kit form.

10. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to GOLLAMUDI S. KISHORE whose telephone number is (571)272-0598. The examiner can normally be reached on 6:30 AM- 4 PM, alternate Friday off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Krass Frederick can be reached on (571) 272-0580. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Gollamudi S Kishore/
Primary Examiner, Art Unit 1612

GSK

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/601,451	11/17/2006	Daryl C. Drummond	HERM1130-2	5211

28213 7590 07/12/2011

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EXAMINER
KISHORE, GOLLAMUDI S

ART UNIT	PAPER NUMBER
1612	

MAIL DATE	DELIVERY MODE
07/12/2011	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Interview Summary	Application No.	Applicant(s)
	11/601,451	DRUMMOND ET AL.
	Examiner	Art Unit
	Gollamudi S. Kishore, PhD	1612

All participants (applicant, applicant's representative, PTO personnel):

(1) *Gollamudi S. Kishore, PhD*.	(3) *Dr. Edward Robinson*.
(2) *Dr. Seth A. Fidel*.	(4)_____.

Date of Interview: *06 July 2011*.

Type: a)☐ Telephonic b)☐ Video Conference
c)☐ Personal [copy given to: 1)☐ applicant 2)☒ applicant's representative]

Exhibit shown or demonstration conducted: d)☐ Yes e)☒ No.
If Yes, brief description: _____.

Claim(s) discussed: *Claims on record*.

Identification of prior art discussed: *Prior art on record*.

Agreement with respect to the claims f)☐ was reached. g)☒ was not reached. h)☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: *See Continuation Sheet*.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN A NON-EXTENDABLE PERIOD OF THE LONGER OF ONE MONTH OR THIRTY DAYS FROM THIS INTERVIEW DATE, OR THE MAILING DATE OF THIS INTERVIEW SUMMARY FORM, WHICHEVER IS LATER, TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached sheet.

/Gollamudi S. Kishore/ Primary Examiner, Art Unit 1612		

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

Continuation of Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: The invention was discussed. Applicant will limit the claims to specific liposome composition containing either irinotecan or topotecan and liposomes containing specific phospholipids and sucrose octasulfate or insositol hexaphosphate; Dr. Fidel discussed the unexpected properties of these liposomes compared to others; Since the application is in final state, applicant will file a RCE and the examiner will carefully evaluate the unexpected properties and determine the allowability of the claims after a search for the specific components in the liposomes. .

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/416,204	03/09/2012	Keelung Hong	89255-826163	3217

20350 7590 05/08/2012

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EXAMINER	
SHOMER, ISAAC	

ART UNIT	PAPER NUMBER
1612	

NOTIFICATION DATE	DELIVERY MODE
05/08/2012	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

Docket@kilpatricktownsend.com
ipefiling@kilpatricktownsend.com
jlhice@kilpatrick.foundationip.com

Notice of References Cited	Application/Control No. 13/416,204	Applicant(s)/Patent Under Reexamination HONG ET AL.	
	Examiner ISAAC SHOMER	Art Unit 1612	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-5,783,568 A	07-1998	Schlessinger et al.	514/53
*	B	US-2002/0102298 A1	08-2002	Needham, David	424/450
*	C	US-2002/0192275 A1	12-2002	Zalipsky et al.	424/450
*	D	US-2003/0138481 A1	07-2003	Zadi, Brahim	424/450
*	E	US-8,147,867 B2	04-2012	Hong et al.	424/450
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

First Action Interview Pilot Program Pre-Interview Communication	Application No. 13/416,204	Applicant(s) HONG ET AL.	
	Examiner ISAAC SHOMER	Art Unit 1612	Page 1 of 2

-The MAILING OR NOTIFICATION DATE of this communication appears on the cover sheet with the correspondence address -

THE SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE **ONE MONTH OR THIRTY (30) DAYS,** WHICHEVER IS LONGER, FROM THE MAILING OR NOTIFICATION DATE OF THIS COMMUNICATION.

This time period for reply is extendable under 37 CFR 1.136(a) for only ONE additional MONTH.
This communication constitutes notice under 37 CFR 1.136(a)(1)(i).

Applicant must, within the time period for reply, file: (1) A letter requesting not to have a first action interview; (2) A reply under 37 CFR 1.111 waiving the first action interview and First Action Interview Office Action; or (3) An Applicant Initiated Interview Request Form (PTOL-413A) electronically via EFS-Web, accompanied by a proposed amendment or arguments, and schedule the interview within 2 months from the filing of the request. A failure to respond to this communication will be treated as a request not to have an interview. If applicant waives the First Action Interview Office Action, the instant Pre-Interview Communication is deemed the first Office Action on the Merits. The next subsequent Office action may be made final if appropriate. See MPEP 706.07(a).

Disposition of Claims

3)☒ Claim(s) *1-20* is/are pending in the application.
3a) Of the above claim(s) _____ is/are withdrawn from consideration.
4)☐ Claim(s) _____ is/are allowed.
5)☒ Claim(s) *1-20* is/are rejected.
6)☐ Claim(s) _____ is/are objected to.
7)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

8)☐ The specification is objected to by the Examiner.
9)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
10)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

11)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
a)☐ All b)☐ Some * c)☐ None of:
1.☐ Certified copies of the priority documents have been received.
2.☐ Certified copies of the priority documents have been received in Application No. _____.
3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
*See the attached detailed Office action for a list of the certified copies not received.

Contact Information

Examiner's Telephone Number: (571)270-7671
Examiner's Typical Work Schedule: 8:00 AM - 5:00 PM Monday-Friday
Supervisor's Name: Frederick F. Krass
Supervisor's Telephone Number: (571)272-0580

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *20 April 2012*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

First Action Interview Pilot Program Pre-Interview Communication	Application No. 13416204	Applicant(s) HONG ET AL.	
	Examiner ISAAC SHOMER	Art Unit 1612	Page 2 of

Notification of Rejection(s) and/or Objection(s)

#	Claim(s)	Reference(s) (if applicable)	Rejection Statutory Basis	Brief Explanation of Rejection
1	1, 3-6, 9, 11	Needham, Schlessinger	35 U.S.C. 103	Needham is drawn to a liposome formulation, including paclitaxel. See Needham, 0072. Schlessinger teaches sucrose octasulfate to treat cancer, as of col. 27 line 24. Obvious to combine as they both treat cancer.
2	1-5, 12-20	Hong	Nonstatutory Double Patenting	This is an anticipatory ODP: Patent claim is drawn to liposome with iriontecan (anti-cancer agent) and sucrose octasulfate, and "anticipates" instant claims. This may be overcome with a terminal disclaimer.
3	1-9, 11	Zadi, Schlessinger	35 U.S.C. 103	Zadi teaches liposome comprising paclitaxel and sugar, as of Zadi, abstract. Schlessinger teaches sucrose octasulfate to treat cancer, as of col. 27 line 24. Obvious to combine as they both treat cancer.
4	10	Zalipsky, Needham, Zadi	35 U.S.C. 103	Zalipsky teaches liposome with targeting ligand. Targeting ligand may be antibody, as of Zalipsky, paragraph 0015. Liposome may deliver paclitaxel to treat neoplasms (cancer), as of Zadi, 0090. Obvious to comine with Needham or Zadi to treat cancer.

Expanded Discussion/Commentary

1		Needham teaches pH 7.4 at which both sucrose octasulfate and paclitaxel would be ionized, and as such, they would be a salt. Needham teaches DSPE-PEG as of par. 0059, parenteral admin. as of par. 0081.
3		10% mass ratio of drug:lipid, of Zadi, abstract, this is about a ratio of 0.08 mol drug to 1 mol lipid, reading on claims 2 and 7. No mention of surfactant or cyclodextrin, as of claim 8. Admin. by injection (parenteral) as of Zadi, 0062. PEG lipids, Zadi, 0036.

DATE: 02 May, 2012	/I. S./ Examiner, Art Unit 1612	/Frederick Krass/ Supervisory Patent Examiner Art Unit 1612

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/416,204	03/09/2012	Keelung Hong	89255-826163	3217

20350 7590 06/29/2012

KILPATRICK TOWNSEND & STOCKTON LLP
TWO EMBARCADERO CENTER
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SAN FRANCISCO, CA 94111-3834

EXAMINER	
SHOMER, ISAAC	

ART UNIT	PAPER NUMBER
1612	

MAIL DATE	DELIVERY MODE
06/29/2012	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Applicant-Initiated Interview Summary	Application No.	Applicant(s)	
	13/416,204	HONG ET AL.	
	Examiner	Art Unit	
	ISAAC SHOMER	1612	

All participants (applicant, applicant's representative, PTO personnel):

(1) *ISAAC SHOMER*.

(2) *Patricia Duffy*.

(3) *Ken Weber*.

(4) *Seth Fidel*.

Date of Interview: *25 June 2012*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☒103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *1-20*.

Identification of prior art discussed: *Schlessinger, Yeh*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

See Continuation Sheet.

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☒ Attachment

/Patricia A Duffy/ Primary Examiner, Art Unit 1645	

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

Continuation of Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments:

1) Applicants presented arguments against the prima facie case of obviousness. In these arguments, applicants contended that the skilled artisan would not have used sucrose octasulfate as an anti-cancer agent. Applicants pointed to Yeh et al. (Molecular and Cellular Biology, October 2002, Vol. 22, No. 20, pages 7184-7192). This reference was cited in the notice of allowance of parent case 11/121,294, submitted 4 January 2012, as teaching that sucrose octasulfate can mimic heparin and potentiate cell proliferation. As such, applicant argued that the art is ambiguous as to whether sucrose octasulfate is useful for treating cancer.

2) In regard to the results presented in the specification, applicant contends that the specification shows that the use of sucrose octasulfate in combination with an anti-cancer drug provides superior results in slowing the progression of tumors. Applicants pointed to Figure 32 of the drawings, which shows tumor growth of a mouse treated with free vinorelbine and liposomal vinorelbine with sucrose octasulfate. Applicants pointed to page 79 Table 18 of the specification, which shows increase in the half life of a liposome with topotecan when sucrose octasulfate is present in the liposome. Applicants reminded examiner that data is also shown for liposomes with sucrose octasulfate and irinotecan, as in the parent case. Applicants pointed out that, while irinotecan and topotecan are camptothecin derivatives, vinorelbine is a vinca alkaloid, which is a different class of cationic antineoplastic drug, and for this reason applicants contend that the unexpected properties of a liposome with sucrose octasulfate have been shown for different classes of drugs.

3) In regard to the double patenting rejection, applicants appeared to be willing to submit a terminal disclaimer.

4) At the conclusion of the interview, it was agreed that applicants would submit a formal submission in response to this interview summary.

First Action Interview Office Action Summary	Application No. 13/416,204	Applicant(s) HONG ET AL.	
	Examiner ISAAC SHOMER	Art Unit 1612	Page 1 of 2

The MAILING OR NOTIFICATION DATE of this communication appears on the cover sheet with the correspondence address.

THE SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE **ONE MONTH OR THIRTY (30) DAYS,** WHICHEVER IS LONGER, FROM THE MAILING OR NOTIFICATION DATE OF THIS COMMUNICATION.

This time period for reply is extendable under 37 CFR 1.136(a) for only ONE additional MONTH.

☐ Applicant's request to not have a first-action interview is acknowledged (or the time period for reply set forth in the Pre-Interview Communication has expired and the Office did not receive any reply).

Status

1) ☒ Responsive to communication(s) filed on *31 May, 2012* and interview conducted on *25 June, 2012*.

2) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

3) ☒ Claim(s) *1-20* is/are pending in the application.

3a) Of the above claim(s) _____ is/are withdrawn from consideration.

4) ☐ Claim(s) _____ is/are allowed.

5) ☒ Claim(s) *1-20* is/are rejected.

6) ☐ Claim(s) _____ is/are objected to.

7) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

8) ☐ The specification is objected to by the Examiner.

9) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

10) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

11) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a) ☐ All b) ☐ Some * c) ☐ None of:

1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

Contact Information

Examiner's Telephone Number: (571)270-7671
Examiner's Typical Work Schedule: 8:00 AM - 5:00 PM Monday-Friday
Supervisor's Name: Frederick F. Krass
Supervisor's Telephone Number: (571)272-0580

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

First Action Interview Office Action Summary	Application No. 13416204	Applicant(s) HONG ET AL.	
	Examiner ISAAC SHOMER	Art Unit 1612	Page 2 of 2

Notification of Rejection(s) and/or Objection(s)

#	Claim(s)	Reference(s) (if applicable)	Rejection Statutory Basis	Brief Explanation of Rejection
1	1, 3-6, 9, 11	Needham, Schlessinger	35 U.S.C. 103	Needham is drawn to a liposome formulation, including paclitaxel. See Needham, 0072. Schlessinger teaches sucrose octasulfate to treat cancer, as of col. 27 line 24. Obvious to combine as they both treat cancer.
2	1-5, 12-20	Hong	Nonstatutory Double Patenting	This is an anticipatory ODP: Patent claim is drawn to liposome with iriontecan (anti-cancer agent) and sucrose octasulfate, and "anticipates" instant claims. This may be overcome with a terminal disclaimer.
3	1-9, 11	Zadi, Schlessinger	35 U.S.C. 103	Zadi teaches liposome comprising paclitaxel and sugar, as of Zadi, abstract. Schlessinger teaches sucrose octasulfate to treat cancer, as of col. 27 line 24. Obvious to combine as they both treat cancer.
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Expanded Discussion/Commentary

1		Needham teaches pH 7.4 at which both sucrose octasulfate and paclitaxel would be ionized, and as such, they would be a salt. Needham teaches DSPE-PEG as of par. 0059, parenteral admin. as of par. 0081.
3		10% mass ratio of drug:lipid, of Zadi, abstract, this is about a ratio of 0.08 mol drug to 1 mol lipid, reading on claims 2 and 7. No mention of surfactant or cyclodextrin, as of claim 8. Admin. by injection (parenteral) as of Zadi, 0062. PEG lipids, Zadi, 0036.

DATE:		
25 June, 2012	/I. S./ Examiner, Art Unit 1612	/Patricia Duffy/ Primary Examiner 1645

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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/654,373	10/17/2012	Keelung Hong	89255-853967 (002130US)	6392

20350 7590 08/12/2013

KILPATRICK TOWNSEND & STOCKTON LLP
TWO EMBARCADERO CENTER
EIGHTH FLOOR
SAN FRANCISCO, CA 94111-3834

EXAMINER	
SHOMER, ISAAC	

ART UNIT	PAPER NUMBER
1612	

NOTIFICATION DATE	DELIVERY MODE
08/12/2013	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ipefiling@kilpatricktownsend.com
jlhice@kilpatrick.foundationip.com
mcollins@kilpatricktownsend.com

Office Action Summary	Application No. 13/654,373	Applicant(s) HONG ET AL.	
	Examiner ISAAC SHOMER	Art Unit 1612	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *1-17* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-17* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some * c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.

3) ☒ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	13/654,373	HONG ET AL.	
	Examiner	Art Unit	
	ISAAC SHOMER	1612	

All participants (applicant, applicant's representative, PTO personnel):

(1) *ISAAC SHOMER*. (3)_____.

(2) *Ken Weber (Attorney)*. (4)_____.

Date of Interview: *29 July 2013*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☐ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *1-17*.

Identification of prior art discussed: *Patents 8,147,867 and 8,329,213*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Examiner called representative of applicant to inform that if terminal disclaimers to the '867 and '213 applications were filed, then the application would be in condition for allowance.

In response, representative of applicant stated that in order to best serve applicant's business interests, the terminal disclaimers would not be filed at this time. As such, the examiner informed representative of applicant that a non-final rejection would be sent.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/I. S./ Examiner, Art Unit 1612	

DETAILED ACTION

Non-Statutory Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the reference application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO internet Web site contains terminal disclaimer forms which may be used. Please visit http://www.uspto.gov/forms/. The filing date of the application will determine what form should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 1-4 and 11-20 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-35 of U.S. Patent No. 8,147,867.

Although the claims at issue are not identical, they are not patentably distinct from each other because of the following reasons:

Instant claim 1 is drawn to a method for delivering an antineoplastic agent by injection to a tumor for treating said tumor. Said antineoplastic agent is administered by a liposome, wherein said liposome comprises an aqueous interior space that is separate from the aqueous medium by a lipid layer, and comprises both sucrose octasulfate and a cationic antineoplastic agent.

Conflicting claim 1 is drawn to a liposomal irinotecan composition, comprising a liposome that includes irinotecan and sucrose octasulfate. This liposome is useful for treating a tumor, as of conflicting claims 2 and 3. This composition is administered via parenteral administration, as of conflicting claim 13. The term “parenteral” is defined by the specification of the ‘867 document as including routes of administration such as

injections that are intramuscular, subcutaneous, intravenous, intraarterial, and other routes, as of column 26 lines 50-54 of the ‘867 document.

As such, conflicting claims 1, 2, and 13 recite all of the requirements of instant claim 1, and are thereby understood to effectively anticipate claim 1. When an examined claim is anticipated by the reference claim, this is a prima facie case of non-statutory double patenting, as explained in the above explanation of double patenting.

As to instant claims 2 and 12, this claim requires a specific molar ratio of lipid to drug. Conflicting claim 14 requires a molar ratio of drug to the totality of lipids of at least 1.0.

As to conflicting claim 3, this claim requires a neutral or anionic PEG-lipid derivative. Conflicting claims 25, 26, and 31 teach a PEG derivative including PEG-DSPE.

As to instant claims 4 and 14, the claim requires a fluid pharmaceutical formulation for parenteral administration. This is taught by conflicting claim 13.

As to instant claim 11, this is an independent claim drawn specifically to a method for administering irinotecan via a liposome comprising irinotecan and its sucrose octasulfate salt. This is taught by conflicting claims 1, 2, and 13, as explained above.

As to instant claim 13, this claim requires a molar ratio of at least 0.1. Conflicting claim 14 requires a molar ratio of drug to the totality of lipids of at least 1.0. As this is narrower than the claimed molar ratio, it is understood to read on the claim.

As to instant claim 15, this claim requires a half-release time of at least 24 hours. Such a half-release time is taught by instant claim 1.

As to instant claim 16, this claim requires a half-release time of at least 48 hours. Such a half-release time is taught by conflicting claim 2.

As to instant claim 17, this claim requires that at least 90% of the irinotecan remains in the liposome after storage for 6 months at 4-8 degrees Celsius. Conflicting claim 32 recites a more stringent storage requirement, requiring that less than 5% of irinotecan leak from the liposome after 6 months of storage. As such, claim 17 is understood to anticipate the instant claims.

Claims 1-17 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-17 of U.S. Patent No. 8,329,213.

Although the claims at issue are not identical, they are not patentably distinct from each other because of the following reasons:

Instant claim 1 is drawn to a method for delivering an antineoplastic agent by injection to a tumor for treating said tumor. Said antineoplastic agent is administered by a liposome, wherein said liposome comprises an aqueous interior space that is separate from the aqueous medium by a lipid layer, and comprises both sucrose octasulfate and a cationic antineoplastic agent.

Conflicting claim 1 is drawn to a composition comprising a liposome with an aqueous interior space and a lipid layer, whereby the interior space includes sucrose

octasulfate and a cationic antineoplastic agent. This composition is used for parenteral administration, as of conflicting claim 4. The term “parenteral” is defined by the specification of the ‘213 document as including routes of administration such as injections that are intramuscular, subcutaneous, intravenous, intraarterial, and other routes, as of column 26 lines 50-54 of the ‘213 document.

As such, the subject matter of conflicting claims 1 and 4 appears to anticipate instant claim 1. When an examined claim is anticipated by the reference claim, this is a prima facie case of non-statutory double patenting, as explained in the above explanation of double patenting.

As to instant claims 2, 12, and 13 this claim requires a specific molar ratio of drug to lipids. The required ratios are taught by conflicting claims 2, 12, and 13.

As to instant claim 3, the claim requires a neutral or anionic lipid-PEG derivative. This is taught by conflicting claim 3.

As to instant claims 4, 10, and 14 the claims require a formulation for parenteral administration, which is taught by conflicting claims 4, 10, and 14.

As to instant claim 5, this claim requires a microtubule stabilizing agent, which is taught by taught claim 5.

As to instant claim 6, this claim requires a drug that is a taxane, which is taught by conflicting claim 6.

As to instant claim 7, this claim requires at least 0.05 mole of taxane per mole of lipids, which is taught by conflicting claim 7.

As to instant claim 8, this claim requires that the composition used in the instant method be essentially free of a solubilizing aid such as micelle-forming surfactant or cyclodextrin. This is taught by conflicting claim 8.

As to instant claim 9, this claim requires a targeting moiety that comprises an antigen-binding sequence of an antibody. This is taught by conflicting claim 9.

As to instant claim 11, this is an independent claim drawn specifically to a method for administering irinotecan via a liposome comprising irinotecan and its sucrose octasulfate salt. This is taught by conflicting claim 11.

As to claims 15 and 16, these claims require an irinotecan half-release time of 24 and 48 hours respectively. These half-release times are taught by conflicting claims 15 and 16.

As to claim 17, this claim requires that 90% of the irinotecan remain in the interior space of the liposome after storage for 6 months at 4 to 8 degrees Celsius. This is taught by conflicting claim 17.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ISAAC SHOMER whose telephone number is (571)270-7671. The examiner can normally be reached on 8:00 AM - 5:00 PM Monday-Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Frederick F. Krass can be reached on (571)272-0580. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ISAAC SHOMER/
Examiner, Art Unit 1612

/Frederick Krass/
Supervisory Patent Examiner, Art Unit 1612

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/151,632	01/09/2014	Daryl C. Drummond	239669-372962	7150

133156 7590 04/18/2016

Honigman Miller Schwartz and Cohn LLP
350 East Michigan Avenue
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Kalamazoo, MI 49007

EXAMINER
KISHORE, GOLLAMUDI S

ART UNIT	PAPER NUMBER
1612	

NOTIFICATION DATE	DELIVERY MODE
04/18/2016	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patents@honigman.com
cbott@honigman.com
fhunter@honigman.com

Office Action Summary	Application No. 14/151,632	Applicant(s) DRUMMOND ET AL.	
	Examiner Gollamudi S. Kishore, PhD	Art Unit 1612	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-12* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-12* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

Claims included in the prosecution are 1-12.

Claim Rejections - 35 USC § 103

1. The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained through the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

2. Claims 1-12 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over WO 2004/017940 and/or WO 03/030,864 in combination with Schlessinger (5,783,568) and either Anderson US 2002/0049176 of record), Kaplitt (US 2006/0129126 of record) Gillis (US 2004/0243101 or Nielson (6,350,853) individually or in combination.

WO 2004/017940 teaches liposomes containing SN 38 (which is an active metabolite of irinotecan) for the treatment of brain cancers. The liposomes contain phosphatidylcholine and cholesterol (Abstract, page 3, line 28 through page 4, line 19, pages 5-7, page 12, line 23 and examples).

WO 03 teaches liposomal compositions containing irinotecan for the treatment of cancers (Abstract, Examples and claims).

What is lacking in WO2004 and 03 is the inclusion of sucrose octasulfate and the conduit for delivery into the brain.

Schlessinger teaches a salt or complex of sulfated saccharide for use in the treatment of cancer. According to Schlessinger sucrose octasulfate bind the heparin-binding growth factors-ligands in a monovalent manner and prevent or disrupt the formation of the heparin stabilized ligand-receptor complexes required for dimerization and activation of the receptor involved in the condition treated. The compounds can be administered using liposomes (abstract, col. 6, lines 15-23, col. 7, lines 45-67, col. 11, line 44, col. 12, lines 3-14 and claims).

Anderson while disclosing modulation of mitochondrial mass and function for the treatment of diseases teaches that for the delivery into the brain the composition may be injected into the brain via cannula (0397).

Kaplitt teaches that delivery devices such as liposomes can be injected directly into the brain using a catheter (0003 and claim 1).

Gillis similarly teaches chemotherapeutic agents in liposomes can be delivered to a tumor in the brain of the mammal using a catheter (0032, claims 1 and 7).

Nielsen teaches that liposomal compositions can be directly delivered to the brain using silicon catheter to treat brain diseases (see col. 12, lines 41-64).

It would have been obvious to one of ordinary skill in the art to include sucrose octasulfate in the liposomal composition with the expectation of obtaining at least an additive effect since Schlessinger teaches that sucrose octasulfate could be used for the treatment of cancers. It would have been obvious to one of ordinary skill in the art to inject the liposomal compositions of WO 2004 or 03 directly into the brain with a reasonable expectation of success since Anderson or Kaplitt or Gillis teach that

compositions containing camptothecin can be directly injected into the brain via cannula. Although WO does not teach irinotecan, since it teaches that SN 38 is an active metabolite of irinotecan (which is converted to SN 38 primarily in the liver and 2000 fold more potent than irinotecan), it would have been obvious to one of ordinary skill in the art to use irinotecan since it gets converted to SN38 in the liver for anti-cancer activity with a reasonable expectation of success.

3. Claims 1-12 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over WO 2004 and/or o3/030864 in combination with Schlessinger (5,783,568) and as set forth above, further in view of Daftary (US 2005/0142178) and/or Torchilin (5,534,241) of record.

Daftary while disclosing doxorubicin containing liposomal compositions, teaches that the liposomes can be loaded with diagnostic agents for MRI imaging (Abstract and 0063).

Torchilin discloses liposomal compositions containing chelating groups and labeling ions such as Gadolinium for imaging a target region of the body of the patient (Abstract, col. 5, lines 20-24; col. 7, lines 1-54 col. 9, line 60 through col. 10, line 31; col. 12, lines 31-67 and claims).

The inclusion of a MRI diagnostic agent in the liposomal compositions, if diagnostics are also desired, would have been obvious to one of ordinary skill in the art since Daftary and Torchilin teach that diagnostic agents can be encapsulated in the liposomes.

Double Patenting

1. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the reference application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

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out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

2. Claims 1-12 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-35 of U.S. Patent No. 8,147,867 by itself or in combination with either Anderson US 2002/0049176 of record), Kaplitt (US 2006/0129126 of record) Gillis (US 2004/0243101 or Nielson (6,350,853) individually or in combination. Although the claims at issue are not identical, they are not patentably distinct from each other because instant claims are drawn to a brain conduit adopted for placement within the brain tissue of a mammal containing a liposomal formulation containing sucrose octasulfate and a cationic antineoplastic agent; the patented claims are drawn to a liposomal composition containing a neutral lipid, uncharged lipid and encapsulated irinotecan and sucrose octasulfate. Instant claims are generic with respect to liposomal composition and antineoplastic agent and it would have been obvious to one of ordinary skill in the art to use any antineoplastic agent which is effective in the treatment of brain cancer, if the cancer happens to be in the brain in suitable liposomal formulation. It would have been obvious to one of ordinary skill in the art to use proper means of delivery such as a 'fluid conduit' if the cancer is brain cancer. It would have been obvious to one of ordinary skill in the art to deliver the composition using a catheter to the brain tissue since Anderson, Kaplitt, Gillis or Nielsen teach that such a technique is known in the art to treat brain diseases (see col. 12, lines 41-64 of Nielsen).

3. Claims 1-12 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-22 of U.S. Patent No. 8,658,203. Although the claims at issue are not identical, they are not patentably distinct from each other because instant claims are drawn to a brain conduit adopted for placement within the brain tissue of a mammal containing a liposomal formulation containing sucrose octasulfate and a cationic antineoplastic agent; the patented claims are drawn to a method of treating brain cancer via conduit using a liposomal composition containing a neutral lipid, uncharged lipid and encapsulated irinotecan and sucrose octasulfate. Instant composition is an obvious variant since no restriction was made in the parent case. It would have been obvious to one of ordinary skill in the art to use proper means of delivery such as a 'fluid conduit' if the cancer is brain cancer. It would have been obvious to one of ordinary skill in the art to deliver the composition using a catheter to the brain tissue since Anderson, Kaplitt, Gillis or Nielsen teach that such a technique is known in the art to treat brain diseases.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gollamudi S. Kishore, PhD whose telephone number is (571)272-0598. The examiner can normally be reached on 6:30 AM- 4 PM, alternate Friday off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Krass Frederick can be reached on (571) 272-0580. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/Gollamudi S. Kishore/
Primary Examiner, Art Unit 1612

GSK

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/175,365	02/07/2014	Keelung HONG	89255-897376 (002150US)	3377

20350 7590 06/26/2014

KILPATRICK TOWNSEND & STOCKTON LLP
TWO EMBARCADERO CENTER
EIGHTH FLOOR
SAN FRANCISCO, CA 94111-3834

EXAMINER	
SHOMER, ISAAC	

ART UNIT	PAPER NUMBER
1612	

NOTIFICATION DATE	DELIVERY MODE
06/26/2014	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ipefiling@kilpatricktownsend.com
jlhice@kilpatrick.foundationip.com

Office Action Summary	Application No. 14/175,365	Applicant(s) HONG ET AL.	
	Examiner ISAAC SHOMER	Art Unit 1612	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-22 and 24-26* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-22 and 24-26* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

Notice of Pre-AIA or AIA Status

The present application is being examined under the pre-AIA first to invent provisions.

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

Information Disclosure Statement

The information disclosure statement filed 4 April 2014 has been considered. However, some references have been crossed out either because they have not been provided or because there was no date provided in the reference. The reason for the references being crossed out is explained in detail on the PTO-1449 form itself.

Claim Objections – Claim Numbering

In regard to claim numbering, the examiner notes that claim 23 has been skipped, and the claims are numbered as 20, 21, 22, 24, 25 26. No explanation is

provided for why claim 23 has been skipped (e.g. there is no explanation that the claim is cancelled). For the purposes of examination, the examiner will examine the case as if a claim 23 had been previously presented and is cancelled; the claim numbers used by applicant will be applied to the currently pending claims.

Claim Rejections - 35 USC § 112(d) – Failure to Further Limit Parent Claim

The following is a quotation of 35 U.S.C. 112(d):

> (d) REFERENCE IN DEPENDENT FORMS.—Subject to subsection (e), a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), fourth paragraph:

> Subject to the [fifth paragraph of 35 U.S.C. 112 (pre-AIA)], a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

Claims 2-5 are rejected under 35 U.S.C. 112(d) or pre-AIA 35 U.S.C. 112, 4th paragraph, as being of improper dependent form for failing to further limit the subject matter of the claim upon which it depends, or for failing to include all the limitations of the claim upon which it depends

Claims 2-5 depend from claim 1. Claim 1 recites a substituted ammonium compound; however, it appears that this substituted ammonium compound of claim 1 is optional. As such, claims 2-5 further limit an optional component. However, as the component being limited by claims 2-5 is not required, these claims are not understood to further limit claim 1.

For the purposes of examination under prior art, claims 2-5 are understood to have the same scope as claim 1.

This can be fixed by clearly reciting in the claim that the substituted ammonium compound is required in claims 2-5.

Claim Rejections - 35 USC § 103

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter

of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

Claims 1-9, 11-14, 16-22, and 24-26 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Kirpotin (US Patent 6,110,491) in view of Koch et al. (US Patent 5,538,954).

Kirpotin is drawn to liposomes, comprising an encapsulated compound, as of Kirpotin, title and abstract. The concentration of the compound is "severalfold" (i.e. several orders of magnitude) higher in the liposome than in the bulk medium, as of Kirpotin, title and abstract. Kirpotin provides an extensive list of compounds (i.e. drugs or therapeutic agents) which may be included, as of Kirpotin, column 5 line 53 to column 6 line 38, some of which are cationic drugs. Kirpotin teaches tetracyclines, as of Kirpotin, column 6 line 2. The liposome of Kirpotin includes cholesterol (an uncharged lipid) and phosphatidylcholine (a zwitterionic phospholipid which is neutral and reads on the required neutral phospholipid), as of Kirpotin, column 9 lines 58-65.

Kirpotin does not teach sucrose octasulfate.

Koch et al. (hereafter referred to as Koch) is drawn to a sucrose octasulfate salt of a tetracycline that is useful in inhibiting the synthesis of bacteria, as of Koch, title and

abstract. In one embodiment, Koch teaches doxycycline in the form of a sucrose octasulfate salt, as of Koch, column 5 lines 4-15. The use of the sucrose octasulfate salt results in low solubility, as of Koch, column 4 lines 41 and 42.

Koch does not teach a liposome.

It would have been prima facie obvious for one of ordinary skill in the art to have used sucrose octasulfate as the counter-ion for the [cationic] drugs taught by Kirpotin in the liposome of Kirpotin. Kirpotin teaches that the drug in the liposome is in the form of "precipitated compound" and does not appear to be solubilized, as of Kirpotin, abstract. Kirpotin further includes a "precipitating agent" for the purpose of creating a precipitate, as of Kirpotin, column 4 line 21. As such, the skilled artisan would have been motivated to have used sucrose octasulfate, as of Koch, to have predictably provided an insoluble salt of a cationic drug for predictable incorporation into the liposome of Kirpotin with a reasonable expectation of success.

As to claims 2-5, for the purposes of examination under prior art, it appears that the substituted ammonium compound of claim 1 is optional. As such, claims 2-5 further limit an optional component. However, as the component being limited by claims 2-5 is not required, these claims are not understood to further limit claim 1. As such, the rejection of claim 1 also applies to claims 2-5.

As to claims 1 (part "2)" and 2, the claims required a substituted ammonium compound, and claim 2 modifies said compound. Kirpotin teaches more than one drug that has a cationic ammonium group. Kirpotin teaches both lidocaine and procaine as anesthetic agents, as of Kirpotin, column 6 line 33. For the purpose of rejecting this

claim, lidocaine is understood to read on the required cationic pharmaceutical entity and procaine is understood to read on the required substituted ammonium compound. This also reads on claim 2 because procaine includes a tertiary amine. This protonated tertiary amine reads on claim 2 because the nitrogen atom is bound to four entities; the first entity is a hydrogen atom (which reads on R_1) and the second, third, and fourth entities are two carbon alkyl groups (which read on R_2, R_3, and R_4). Combination of two drugs (lidocaine and procaine) for the same purpose (anesthesiology) is prima facie obvious. See MPEP 2144.06. Similarly, the inclusion of two antibiotics, namely erythromycin and azithromycin, as of Kirpotin, column 5, line 66, would read on both the cationic therapeutic agent and the substituted ammonium compound.

As to claim 6-8, Kirpotin teaches the inclusion of polyethylene glycol derivatized distearoylphosphatidyl ethanolamine (PEG-DSPE), as of Kirpotin, column 9 lines 60-62. This reads on the required polymer conjugated lipid, polyethylene glycol conjugated lipid, and the requirements of claim 8.

As to claim 9, Kirpotin teaches a composition for parenteral use with a pH of 6-8, as of Kirpotin, column 8 lines 37-41. In view of the teaching of the pH, the composition is understood to be liquid and aqueous, and therefore "fluid."

As to claims 11-13, Koch teaches doxycycline and sucrose octasulfate in a manner such that their charges balance (e.g. there is an equal number of negative and positive charges), as of Koch, column 5 lines 4-16. This is understood to read on the required stoichiometric equivalence.

As to claim 14, Kirpotin teaches egg phosphatidylcholine, as of Kirpotin, column 9 lines 59-61. This is understood to read on the required diacylphosphatidylcholine, as the egg phosphatidylcholine comprises two lipophilic acyl groups attached to a phosphate and choline.

As to claim 16, Kirpotin teaches a NaCl-HEPES buffer, as of Kirpotin, column 12 lines 26-29.

As to claim 17, Kirpotin teaches that the concentration of precipitated compound (e.g. drug or pharmaceutical agent) is “severalfold” higher in the liposome than in the bulk medium, as of Kirpotin, abstract.

As to claim 18, Kirpotin teaches various types of drugs such as an anti-helmintic (A form of anti-protozoal), as of Kirpotin, column 5 line 59 and a tetracycline, which is known to be an antimicrobial antibiotic, as of Kirpotin, column 6 line 2.

As to claim 19, Kirpotin teaches a tetracycline, which is known to be an antimicrobial antibiotic, as of Kirpotin, column 6 line 2. Koch also teaches doxycycline, which is an antimicrobial antibiotic.

As to claim 20, Kirpotin teaches a long list of drugs as of Kirpotin, column 5 line 53 to column 6 line 38. One such drug taught by Kirpotin is the anti-protozoal drug “pyrantel” as if Kirpotin, column 5 line 59. Pyrantel would have been understood by one of ordinary skill in the art to be cationic because it has a chemical structure including a non-aromatic six membered ring with one double bond, whereby there is a tertiary nitrogen atom that is sp^3 hybridized, this nitrogen atom would have been protonated at neutral pH and therefore pyrantel would have been a cationic pharmaceutical entity.

As to claim 21, this is an independent claim requiring a liposome comprising an aqueous interior space separated from the aqueous medium by a membrane, and containing sucrose octasulfate polyanion and a cationic antimicrobial or antiprotozoal agent. Kirpotin teaches a liposome comprising a membrane and aqueous interior space, as well as both antimicrobial tetracycline and anti-protozoal agents, as explained in the rejection of claims 1, and 18-20 above. Koch teaches sucrose octasulfate as used as a counter-ion for doxycycline. See the explanations above regarding claims 1 and 18-20.

As to claim 22, Kirpotin teaches loading at 200 nanomoles per micromole of liposomal phospholipid, as of Kirpotin, column 12 line 25. This is a molar ratio of 0.2 moles of agent per mole of lipid, and reads on the required at least about 0.05, at leats about 0.1, and at least about 0.2.

As to claim 24, Kirpotin teaches the inclusion of polyethylene glycol derivatized distearoylphosphatidyl ethanolamine (PEG-DSPE), as of Kirpotin, column 9 lines 60-62. This reads on the required neutral PEG-lipid derivative.

As to claim 25, Koch teaches doxycycline and sucrose octasulfate in the form of a salt, as of Koch, column 5 lines 8-11.

As to claim 26, Kirpotin teaches a composition for parenteral use with a pH of 6-8, as of Kirpotin, column 8 lines 37-41. In view of the teaching of the pH, the composition is understood to be liquid and aqueous, and therefore "fluid."

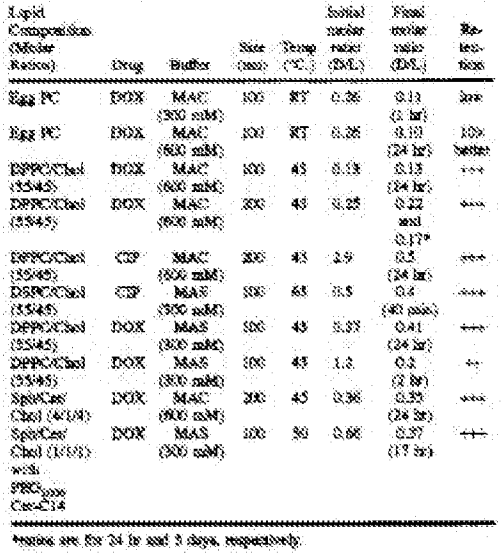
Claims 10 and 15 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Kirpotin (US Patent 6,110,491) in view of Koch et al. (US Patent 5,538,954), the combination further in view of Hope et al. (US Patent 5,785,987).

Kirpotin is drawn to a liposome comprising a drug, wherein the concentration of the drug in the liposome exceeds the concentration of the drug outside the liposome. Koch teaches the use of sucrose octasulfate as a counter-ion for doxycycline. See the rejection above over Kirpotin and Koch by themselves.

Neither Kirpotin nor Koch teach a molar ratio of pharmaceutical entity to lipid of at least 1.0.

Hope et al. (hereafter referred to as Hope) is drawn to preparation of stable liposome formulations of protonatable therapeutic agents. Hope teaches the use of a methylamine concentration gradient to load therapeutic agents across the lipid bilayer of liposomes, as of Hope, column 2 lines 13-18. In one embodiment, Hope achieves molar ratios of drug to lipid as great as 2.9:1, as of Hope, column 20, Table 1, reproduced below.

TABLE 1



It would have been prima facie obvious for one of ordinary skill in the art to have used a drug to lipid loading ratio of up to 2.9, as taught by Hope, in the liposome of Kirpotin in view of Koch. The skilled artisan would have been motivated to have used the loading method of Hope to have predictably increased the amount of drug that could have been loaded into a liposome. The skilled artisan would have been motivated to have done so in order to have predictably increased the amount of drug that can be delivered to a patient with a reasonable expectation of success.

As to claim 15, Hope teaches the use of distearoyl phosphatidylcholine (DSPC) in the liposome, as of Hope, column 5 line 30.

Claim 15 is rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Kirpotin (US Patent 6,110,491) in view of Koch et al. (US Patent 5,538,954), the combination further in view of Barenholz et al. (US Patent 5,192,549).

Kirpotin is drawn to a liposome comprising a drug, wherein the concentration of the drug in the liposome exceeds the concentration of the drug outside the liposome. Koch teaches the use of sucrose octasulfate as a counter-ion for doxycycline. See the rejection above over Kirpotin and Koch by themselves.

Neither Kirpotin nor Koch teach distearoyl phosphatidylcholine (DSPC).

Barenholz et al. (hereafter referred to as Barenholz) is drawn to liposomes in which drugs are loaded, as of Barenholz, title and abstract. The liposomes of Barehnolz include phospholipids, as of Barenholz, column 8 lines 21-32, wherein the phospholipids may include both egg phosphatidylcholine and DSPC. The liposomes may be formed from vesicle forming lipids which may be phospholipids, as of Barenholz, column 8 lines 14-16.

Barenholz does not teach sucrose octasulfate.

It would have been prima facie obvious for one of ordinary skill in the art to have substituted DSPC in place of sucrose octasulfate in the liposome of Kirpotin. Kirpotin teaches a liposome that is made of egg phosphatidylcholine, as of Kirpotin, column 12 line 11. As both DSPC and egg phosphatidylcholine are useful for making liposomes, the skilled artisan would have been motivated to have substituted DSPC in place of egg phosphatidylcholine for predictable use in making a liposome bilayer with a reasonable expectation of success. The simple substitution of one known element (DSPC) in place of another (egg phosphatidylcholine) to achieve predictable results (making a liposome bilayer) is prima facie obvious. See MPEP 2143, Exemplary Rationale B.

In the alternative, the skilled artisan would have been motivated to have used the DSPC of Barenholz to have made the liposome of Kirpotin as both DSPC and egg phosphatidylcholine are taught as useful as liposome phospholipids. Generally, it is *prima facie* obvious to select a known material (DSPC) for incorporation into a composition (a liposome), based on its recognized suitability for its intended use (a phospholipid that forms the bilayer). See MPEP 2144.07.

Non-Statutory Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the reference application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO internet Web site contains terminal disclaimer forms which may be used. Please visit http://www.uspto.gov/forms/. The filing date of the application will determine what form should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 1-17 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-35 of U.S. Patent No. 8,147,867. Although the claims at issue are not identical, they are not patentably distinct from each other because of the following reasons.

Instant claim 1 is drawn to a liposomal composition comprising a liposome having an interior space separated from the aqueous medium by a membrane having an uncharged lipid component and a neutral (i.e. no net charge) phospholipid. Entrapped inside the liposomes are a cationic pharmaceutical entity and sucrose octasulfate, with

an optional substituted ammonium compound. The concentration of the pharmaceutical entity inside the liposomes exceeds its concentration outside the liposomes.

Conflicting claim 1 is drawn to a liposomal irinotecan composition. Said liposomes have an interior space separated from the aqueous medium by a membrane having an uncharged lipid component and a neutral (i.e. no net charge) phospholipid. Entrapped inside the liposomes are irinotecan and sucrose octasulfate, with an optional substituted ammonium compound. Said liposomes have a half-release time of irinotecan of at least 24 hours.

As irinotecan is a cationic pharmaceutical agent, the subject matter of conflicting claim 1 effectively anticipates that of instant claim 1. A nonstatutory double patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim (i.e. instant claim) is not patentably distinct from the reference claim(s) (i.e. conflicting claim) because the instant claim is either anticipated by, or would have been obvious over, the conflicting claim. See the above-reproduced form paragraph prior to the statement of rejection. As conflicting claim 1 effectively anticipates instant claim 1, this results in a prima facie case of non-statutory double patenting.

As to the dependent claims, the following table (on the next page) explains where the teachings of the dependent claims are taught by the claims of the '867 patent.

Instant Claim	Subject Matter	Conflicting Claim
2	Various ammonium nitrogen substituents	9
3	Specific cationic ammonium ions	10
4	Triethylammonium	11
5	Diethylammonium	23
6	Polymer-conjugated lipid	12
7	Polyethylene glycol conjugated lipid	25
8	PEG-DSPE	26
9	Formulation for parenteral administration	13
10	Drug:lipids > 1.0	14
11/12	Stoichiometric Equivalence (drug:sucrose octasulfate)	17
14	Diacylphosphatidylcholine	20
15	DSPC	21
16	HEPES and NaCl in aqueous medium	22
17	Pharmaceutical entity removed from aqueous medium	Would have been obvious from claim 1

As to claim 17, while the conflicting claims do not recite that the concentration of the pharmaceutical entity is partially or substantially removed from outside the liposome,

the skilled artisan would have understood that the purpose of a liposome is for drug delivery, and this cannot occur unless the drug is in the liposome. As such, the skilled artisan would have been motivated to have included the drug in the liposome and removed the drug from outside the liposome to have delivered the drug effectively and in the absence of side effects.

Claims 1, 6, 7, 9, 10, and 17 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-17 of U.S. Patent No. 8,329,213. Although the claims at issue are not identical, they are not patentably distinct from each other because of the following reasons.

Instant claim 1 is drawn to a liposomal composition comprising a liposome having an interior space separated from the aqueous medium by a membrane having an uncharged lipid component and a neutral (i.e. no net charge) phospholipid. Entrapped inside the liposomes are a cationic pharmaceutical entity and sucrose octasulfate, with an optional substituted ammonium compound. The concentration of the pharmaceutical entity inside the liposomes exceeds its concentration outside the liposomes.

Conflicting claim 1 is drawn to a liposome having an aqueous interior space, which is separated from the aqueous medium by a membrane comprised of one or more lipids. This liposome contains sucrose octasulfate as a polyanion and an acid or salt of a cationic neoplastic agent.

The cationic antineoplastic agent of conflicting claim 1 is understood to read on the required cationic pharmaceutical entity. While the conflicting claims do not recite that the concentration of the pharmaceutical entity inside the liposomes exceeds that outside the liposomes, the skilled artisan would have understood that the purpose of a liposome is for drug delivery, and this cannot occur unless the drug is in the liposome. As such, the skilled artisan would have been motivated to have included the drug in the liposome as required by claim 1.

As to the dependent claims, the following table explains where the teachings of the dependent claims are taught by the claims of the ‘213 patent.

Instant Claim	Subject Matter	Conflicting Claim
6	Polymer-conjugated lipid	3
7	Polyethylene glycol conjugated lipid	3
9	Formulation for parenteral administration	4
10	Drug:lipids > 1.0	2
17	Pharmaceutical entity removed from aqueous medium	Would have been obvious from claim 1

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ISAAC SHOMER whose telephone number is (571)270-7671. The examiner can normally be reached on 8:00 AM - 5:00 PM Monday-Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Frederick F. Krass can be reached on (571)272-0580. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ISAAC SHOMER/
Examiner, Art Unit 1612

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/406,776	12/10/2014	Eliel Bayever	239669-373010	6881

133156 7590 02/26/2016

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EXAMINER
STRONG, TORI

ART UNIT	PAPER NUMBER
1629	

MAIL DATE	DELIVERY MODE
02/26/2016	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 14/406,776	Applicant(s) BAYEVER ET AL.	
	Examiner TORI M. STRONG	Art Unit 1629	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *03 September 2015*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *28-45* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *28-45* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☒ The drawing(s) filed on *10 December 2014* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

Notice of Pre-AIA or AIA Status

The present application is being examined under the pre-AIA first to invent provisions.

Status of Claims

Claims 28-45 are pending in the instant application and are the subject of the Office Action below.

Information Disclosure Statement

The information disclosure statements (IDSs) submitted on 03/19/2015, 09/03/2015, 09/17/2015, 09/21/2015, 09/22/2015, 10/15/2015 and 01/11/2016 were filed after the mailing date of the application on December 10, 2014. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner. Enclosed with this Office Action are return copies of Form PTO/SB/08B with the Examiner's initials and signature indicating those references that have been considered.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of pre-AIA 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 28-45 are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Chen *et al.* (*Journal of Clinical Oncology*, 2008, Vol. 26, No. 15S, p. 2565; cited in IDS).

Applicant's invention, according to **claims 28, 34, 38 and 40**, is directed to a method of treating pancreatic cancer in a human patient refractory to gemcitabine therapy, comprising administering 60-180 mg/m^2 of irinotecan in a liposome-encapsulated form having a diameter of about 80-140 nm, having a half-life of about 21-48 hours and comprises phosphatidylcholine, cholesterol, and a polyethyleneglycol (PEG)-derivatized phosphatidyl-ethanolamine. It is noted that the limitations for the liposomal form of irinotecan is also referred to as MM-398. As reasonably interpreted from the specification, MM-398 has all the properties express within the limitations instantly claimed for the liposomal irinotecan (see the paragraph spanning pp. 8 and 9).

Chen teaches treatment of advanced refractory solid tumors where the pancreatic cancer is an exemplified embodiment and the patients are refractory to standard chemotherapy. Chen teaches the liposomal formulation of irinotecan referred to as PEP02, which is the same as MM-398, as evidenced by Tsai *et al.* (*Journal of Gastrointestinal Oncology*, 2011, Vol. 2, pp. 185-194; cited in IDS) (see Tsai *et al.*, p.189, col.2, para.2). Chen teaches administering doses of PEP02 at 60, 120 and 180 mg/m^2, thus meeting the instantly claimed limitation of dose range. Chen teaches the

improvements of PEP02 (or MM-398) over the free form, where the terminal half-life is about 29.5 hours; falling within the instantly claimed range. While Chen does not explicitly point out the features of the liposomal encapsulation, Chen utilizes the same form of irinotecan as instantly disclosed but referred to by a different name. Furthermore, Chen does not explicitly disclose that the refractory therapy is refractory to gemcitabine therapy, however, Chen selects patients that are refractory to standard chemotherapy; and as also evidenced by Tsai *et al.*, gemcitabine, alone or in combination, is the only approved standard treatment for patients with advanced pancreatic cancer (see Tsai *et al.*, p.185, para.1). Therefore Chen is teaching advanced pancreatic cancer refractory to gemcitabine therapy. The instant claims read upon the art of Chen and therefore are anticipated.

Applicant's invention, according to **claims 29-33, 36, 37, 41, 42, 44 and 45**, limits claims 28 and 40 and requires the following: 1) the liposome to comprise of one PEG molecule for 200 phospholipid molecules; 2) the C_{max} of SN-38 at about 7.98 $\pm$ 4.39 ng/mL; 3) the $t_{1/2}$ of SN-38 at about 53.75 $\pm$ 15.6 hours; 4) the AUC of SN-38 at about 502.15 $\pm$ 153 ng.h/mL; and 5) the amount of SN-38 released increases less than proportionally with the dose of MM-398.

As expressed *supra*, the liposome formulation limitations are broadly and reasonably interpreted as the same formulation for MM-398, which is the same as PEP02. Unless evidence to the contrary, the limitation of one PEG molecule for 200 phospholipid will be interpreted as the routine and conventional formulation for MM-398.

Chen teaches the use of PEP02 for treating advanced pancreatic cancer refractory to therapy. Chen also teaches about the active metabolite SN-38; disclosing a C_{max} of about 9.2 $\pm$ 3.5 ng/mL, a $t_{1/2}$ of about 75.4 $\pm$ 43.8 hours, an AUC at about 710 $\pm$ 395 ng.h/mL and further disclosing "...that the release of irinotecan from the liposomes occurred slowly over time." When looking at the statistical standard deviation of the values claimed for the SN-38 profile, the values instantly claimed all fall within the scope of what is disclosed by Chen for the SN-38 profile. Chen's teaching of the irinotecan slowly releasing also meets the limitation of its active metabolite increasing slowly compared to the dose of the liposome form. The instant limitations read upon the art of Chen and therefore the instant claims are anticipated.

Applicant's invention, according to **claims 35, 39 and 43**, limits claims 28 and 40 and requires obtaining a liposomal encapsulated form of irinotecan providing about 5 mg/mL of irinotecan HCl where claim 43 further requires it in a 500 mL of a 5% dextrose injection.

As expressed *supra*, the liposome formulation limitations are broadly and reasonably interpreted as the same formulation for MM-398, which is the same as PEP02. Unless evidence to the contrary, the limitation of a liposomal encapsulated form of irinotecan providing about 5 mg/mL of irinotecan HCl and further formulated in a 500 mL of a 5% dextrose injection will be interpreted as the routine and conventional formulation for MM-398.

Chen teaches the use of PEP02 for treating advanced pancreatic cancer refractory to therapy. Therefore the instant limitations read upon the art of Chen as broadly and reasonably interpreted and therefore the instant claims are anticipated.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the reference application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP §

717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/forms/. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 28-45 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claim 1-30 of copending Application No. 14/844,500, over claims 1-20 of copending Application No. 14/851,111 and over claims 1-12, 14, 15 and 20-32 of allowed (but not issued) Application No. 14/812,950. Although the claims at issue are not identical, they are not patentably distinct from each other because each of the applications set out to claim a method of treating pancreatic cancer refractory to gemcitabine therapy through intravenous administration of composition comprising irinotecan as the MM-398 liposome. All the applications claim the method of administering MM-388 at an identical dose where the regimen is administered in a two week cycle. The claims of the copending applications are obvious variants of each

other. This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to TORI M. STRONG whose telephone number is (571)272-6333. The examiner can normally be reached on Monday-Friday 8am-5pm EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey Lundgren can be reached on 571-272-5541. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/TORI M STRONG/
Examiner, Art Unit 1629

/Kortney L. Klinkel/
Primary Examiner, Art Unit 1611

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Eliel Bayever, et al.

Application No.: 14/406,776

Confirmation No.: 6881

Filed: December 10, 2014

Art Unit: 1629

For: METHODS FOR TREATING PANCREATIC CANCER USING COMBINATION THERAPIES COMPRISING LIPOSOMAL IRINOTECAN

Examiner: Tori Strong

Mail Stop Amendments
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT IN RESPONSE TO NON-FINAL OFFICE ACTION DATED FEBRUARY 26, 2016

Dear Madam:

In response to the Non-Final Office action, dated February 26, 2016 ("Office Action"), please amend the application as follows and consider the remarks set forth below.

Amendments to the Claims are reflected in the listing of claims which begin on page 2 of this paper.

Remarks/Arguments begin on page 9 of this paper.

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application. Please amend the claims as follows.

Listing of Claims

1.-27. (Canceled).

28. (Currently Amended) A method of treating pancreatic cancer in a human patient who has previously been treated with gemcitabine, the method comprising intravenously administering to the patient in need thereof ~~60-180 mg/m^2 of irinotecan in a liposome-encapsulated irinotecan having a diameter of about 80-140 nm and a half-life in the patient of about 21 to 48 hours, wherein the liposome comprises phosphatidylcholine, cholesterol, and a polyethyleneglycol-derivatized phosphatidyl-ethanolamine.~~ an antineoplastic therapy once every two weeks, the antineoplastic therapy consisting of:

a. 60-80 mg/m^2 of liposomal irinotecan composition comprising irinotecan liposomes, the irinotecan liposomes in the liposomal irinotecan composition having a diameter of approximately 80-140 nm and an irinotecan terminal elimination half-life in the patient of about 21-48 hours, wherein the irinotecan liposomes comprise phosphatidylcholine, cholesterol, and a polyethyleneglycol-derivatized phosphatidyl-ethanolamine;

b. 200 mg/m^2 of the (l) form of leucovorin; and

c. 2,400 mg/m^2 of 5-flourouracil.

29. (Currently Amended) The method of claim 28, wherein the irinotecan liposomes comprise ~~liposome comprises~~ approximately one polyethyleneglycol (PEG) molecule for every 200 phospholipid molecules.

30. (Cancelled)

31. (Cancelled)

32. (Cancelled)

33. (Currently Amended) The method of claim 28, wherein the irinotecan is converted to SN-38 and the AUC ~~amount~~ of total SN-38 ~~from irinotecan released from the liposome within the patient~~ increases less than proportionally with the dose of the ~~liposome-encapsulated~~ liposomal irinotecan.

34. (Currently Amended) The method of claim 28, wherein the patient has failed prior treatment with gemcitabine or become resistant to gemcitabine prior to administration of the ~~liposome-encapsulated~~ liposomal irinotecan composition.

35. (Cancelled)

36. (Cancelled)

37. (Currently Amended) The method of claim 34 [[36]], wherein the irinotecan liposomes comprise ~~liposome comprises~~ approximately one polyethyleneglycol (PEG) molecule for every 200 phospholipid molecules.

38. (Cancelled)

39. (Cancelled)

40. (Currently Amended) A method of treating pancreatic cancer in a human patient who has previously been treated with gemcitabine, the method comprising intravenously administering to the patient in need thereof an antineoplastic therapy comprising one or more two-week treatment cycles, each two-week treatment cycle consisting of the administration, starting on the first day of each two-week treatment cycle of: ~~60-80 mg/m^2 of irinotecan as a liposome-encapsulated irinotecan having a diameter of about 80-140 nm and a half-life in the patient of about 21 to 48 hours,~~

~~wherein the liposome comprises phosphatidylcholine, cholesterol, and a polyethyleneglycol-derivatized phosphatidyl-ethanolamine.~~

a. 60-80 mg/m^2 of liposomal irinotecan composition comprising irinotecan liposomes, the irinotecan liposomes in the liposomal irinotecan composition having a diameter of approximately 80-140 nm and an irinotecan terminal elimination half-life in the patient of about 21 to 48 hours, wherein the irinotecan liposomes comprise irinotecan encapsulated in a unilamellar lipid bilayer vesicle composed of phosphatidylcholine, cholesterol, and a polyethyleneglycol-derivatized phosphatidyl-ethanolamine; the irinotecan liposomes administered in combination with

b. 200 mg/m^2 of the (l) form of leucovorin; and

c. 2,400 mg/m^2 of 5-fluorouracil.

41. (Currently Amended) The method of claim 40, wherein the irinotecan liposomes comprise ~~liposome comprises~~ approximately one polyethyleneglycol (PEG) molecule for every 200 phospholipid molecules.

42. (Cancelled)

43. (Cancelled)

44. (Currently Amended) A method of treating pancreatic cancer in a human patient who has previously been treated with gemcitabine, the method comprising intravenously administering to the patient in need thereof an antineoplastic therapy once every two weeks, the antineoplastic therapy consisting of: ~~60-80 mg/m^2 of irinotecan in a liposome-encapsulated irinotecan having a diameter of about 80-140 nm and a half-life in the patient of about 21 to 48 hours, wherein the liposome comprises phosphatidylcholine, cholesterol, and a polyethyleneglycol-derivatized phosphatidyl-ethanolamine, wherein the irinotecan is released from the liposome within the patient and converted to SN-38 within the patient and the amount of SN-~~

~~38 from irinotecan released from the liposome within the patient increases less than proportionally with the dose of the liposome-encapsulated irinotecan.~~

a. 60, 70 or 80 mg/m^2 of liposomal irinotecan composition comprising irinotecan liposomes, the irinotecan liposomes in the liposomal irinotecan composition having a diameter of about 80-140 nm and an irinotecan terminal elimination half-life in the patient of about 21 to 48 hours, wherein the irinotecan liposomes comprise irinotecan encapsulated in a unilamellar lipid bilayer vesicle composed of phosphatidylcholine, cholesterol, and a polyethyleneglycol-derivatized phosphatidyl-ethanolamine; and then administering

b. 200 mg/m^2 of the (l) form of leucovorin over 30 minutes; and then administering

c. 2,400 mg/m^2 of 5-fluorouracil over 46 hours;

wherein the irinotecan is converted to SN-38 within the human patient and the AUC of the SN-38 increases less than proportionally with the dose of the liposomal irinotecan.

45. (Cancelled)

46. (New) The method of claim 28, wherein the 200 mg/m^2 of the (l) form of leucovorin is provided by administering 400 mg/m^2 of the (l+d) form of leucovorin.

47. (New) The method of claim 46, wherein administration of liposomal irinotecan is administered as an infusion over 90 minutes.

48. (New) The method of claim 47, wherein after the irinotecan is administered: the leucovorin is administered over 30 minutes and the 5-fluorouracil is administered over 46 hours.

49. (New) The method of claim 48, wherein the dose of liposomal irinotecan is 80 mg/m^2 and the human patient is not homozygous for the UGT1A1*28 allele.

50. (New) The method of claim 40, wherein the 200 mg/m^2 of the (l) form of leucovorin is provided by administering 400 mg/m^2 of the (l+d) form of leucovorin.

51. (New) The method of claim 50, wherein the irinotecan is administered in an infusion over 90 minutes, the leucovorin is administered after the irinotecan over 30 minutes , and the 5-fluorouracil is administered after the leucovorin over 46 hours.

52. (New) The method of claim 51, wherein the dose of liposomal irinotecan is 80 mg/m^2, and the human patient is not homozygous for the UGT1A1*28 allele.

53. (New) The method of claim 44, wherein the 200 mg/m^2 of the (l) form of leucovorin is provided by administering 400 mg/m^2 of the (l+d) form of leucovorin.

54. (New) The method of claim 53, wherein the irinotecan is administered in an infusion over 90 minutes, the leucovorin is administered after the irinotecan over 30 minutes and the 5-fluorouracil is administered after the leucovorin over 46 hours.

55. (New) The method of claim 54, wherein the antineoplastic therapy comprises at least three two-week treatment cycles.

56. (New) A method of treating pancreatic cancer in a human patient who has previously been treated with gemcitabine, the method comprising intravenously administering to the patient in need thereof an antineoplastic therapy once every two weeks, the antineoplastic therapy consisting of: a single dose of 60, 70 or 80 mg/m^2 of a liposomal irinotecan composition comprising irinotecan liposomes, administered in combination with 200 mg/m^2 of the (l) form of leucovorin and 2,400 mg/m^2 of 5-fluorouracil, to treat the pancreatic cancer in the human patient.

57. (New) The method of claim 56, wherein the irinotecan liposomes have a diameter of about 80-140 nm and an irinotecan terminal elimination half-life in the patient of at least about 2- fold higher than that of 125 mg/m^2 free irinotecan as CPT-11 irinotecan hydrochloride injection.

58. (New) The method of claim 56, wherein the irinotecan liposomes comprise irinotecan encapsulated in a unilamellar lipid bilayer vesicle composed of phosphatidylcholine, cholesterol, and a polyethyleneglycol-derivatized phosphatidyl-ethanolamine, and the 200 mg/m^2 of the (l) form of leucovorin is provided by administering 400 mg/m^2 of the (l+d) form of leucovorin.

59. (New) The method of claim 58, wherein the irinotecan liposomes have an irinotecan terminal elimination half-life in the patient of about 21 to 48 hours.

60. (New) The method of claim 59, wherein the irinotecan is converted to SN-38 within the human patient and the AUC of the SN-38 increases less than proportionally with the dose of the liposomal irinotecan.

61. (New) The method of claim 40, wherein the irinotecan is converted to SN-38 within the human patient and the AUC of the SN-38 increases less than proportionally with the dose of the liposomal irinotecan.

62. (New) The method of claim 28, wherein 80 mg/m^2 of liposomal irinotecan is administered to a human patient who is not homozygous for the UGT1A1*28 allele, and the 5-fluorouracil is administered over 46 hours starting on the first day of each two week treatment cycle.

63. (New) The method of claim 40, wherein 80 mg/m^2 of liposomal irinotecan is administered to a human patient who is not homozygous for the UGT1A1*28 allele.

64. (New) The method of claim 44, wherein 80 mg/m^2 of liposomal irinotecan is administered to a human patient who is not homozygous for the UGT1A1*28 allele.

65. (New) The method of claim 56, wherein 80 mg/m^2 of liposomal irinotecan is administered to a human patient who is not homozygous for the UGT1A1*28 allele.

66. (New) The method of claim 60, wherein 80 mg/m^2 of liposomal irinotecan is administered to a human patient who is not homozygous for the UGT1A1*28 allele, and the method comprises administering the liposomal irinotecan over a 90 minute

infusion, followed by administering 400 mg/m^2 of the (l+d) form of leucovorin over 30 minutes, followed by administering the 5-fluorouracil over 46 hours.

67. (New) The method of claim 40, wherein the patient has failed prior treatment with gemcitabine or become resistant to gemcitabine prior to administration of the liposomal irinotecan composition.

68. (New) The method of claim 44, wherein the patient has failed prior treatment with gemcitabine or become resistant to gemcitabine prior to administration of the liposomal irinotecan composition.

69. (New) The method of claim 56, wherein the patient has failed prior treatment with gemcitabine or become resistant to gemcitabine prior to administration of the liposomal irinotecan composition.

70. (New) The method of claim 28, wherein the antineoplastic therapy comprises at least three two-week treatment cycles.

71. (New) The method of claim 40, wherein the antineoplastic therapy comprises at least three two-week treatment cycles.

72. (New) The method of claim 56, wherein the antineoplastic therapy comprises at least three two-week treatment cycles.

REMARKS

Claim Status

Currently, claims 28, 29, 33, 34, 37, 40, 41, 44, and 46-72 are pending in this application. Claims 30-32, 35, 36, 38, 39, 42, 43 and 45 have been cancelled without prejudice or disclaimer. Claims 28, 29, 33, 34, 37, 40, 41 and 44 have been amended. New claims 46-72 are added. The claim amendments and new claims are supported in the specification as filed; no new matter is added. With the claim amendments, a total of 35 claims with 4 independent claims remain pending.

The amendments to and/or cancellation of the claims are being made for the purpose of expediting prosecution and to place the application in better condition for appeal, should an appeal be necessary, and are made without prejudice or waiver.

This listing of claims will replace all prior versions and listings of claims in the application. Applicant reserves the right to present the original claims in this or a continuing application.

Claim Rejections – 35 USC § 102

The Examiner has rejected claims 28-45 under pre-AIA 35 U.S.C. § 102(b) in the Office Action as allegedly being anticipated by Chen *et al., Journal of Clinical Oncology,* 2008, Vol. 26, No. 15S, p. 2565 ("Chen").

Applicant respectfully disagrees with the rejection. To anticipate a claim under 35 U.S.C. § 102, a single prior art reference must disclose each and every limitation "arranged as in the claim," and enable the claimed invention. See Structural Rubber Prods. Co. v. Park Rubber Co., 749 F.2d 707, 716 (Fed. Cir. 1984); and Vizio, Inc. v. ITC, 605 F.3d 1330, 1342 (Fed. Cir. 2010) ("An anticipatory reference must show all of the limitations of the claims combined in the same way as recited in the claims") (emphasis added). Anticipation also requires that the reference must disclose the invention "without any need for picking, choosing, and combining various disclosures...." In re Arkley, 455 F.2d 586, 587-88 (CCPA 1972). There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention. See Scripps Clinic & Res. Found. V. Genentech, Inc., 927 F.2d 1565, 1576 (Fed. Cir. 1991).

With entry of this Response, independent claim 28 reads:

> A method of treating pancreatic cancer in a human patient who has previously been treated with gemcitabine, the method comprising intravenously administering to the patient in need thereof an antineoplastic therapy once every two weeks, the antineoplastic therapy consisting of: a. 60-80 mg/m^2 of liposomal irinotecan composition comprising irinotecan liposomes, the irinotecan liposomes in the liposomal irinotecan composition having a diameter of approximately 80-140 nm and an irinotecan terminal elimination half-life in the patient of about 21-48 hours, wherein the irinotecan liposomes comprise phosphatidylcholine, cholesterol, and a polyethyleneglycol-derivatized phosphatidyl-ethanolamine; b. 200 mg/m^2 of the (l) form of leucovorin; and c. 2,400 mg/m^2 of 5-flourouracil.

Each of claims 29, 33, 34, and 37 depends from claim 28, and thus incorporates the new limitation added to claim 28 by this amendment. The Office Action does not indicate how Chen anticipates independent claim 28. Applicants respectfully submit that Chen does not disclose the methods covered by either independent claim 28, nor the rejected claims depending therefrom. Reconsideration and withdrawal of this rejection are requested.

With entry of this Response, claim 40 reads:

> A method of treating pancreatic cancer in a human patient who has previously been treated with gemcitabine, the method comprising intravenously administering to the patient in need thereof an antineoplastic therapy comprising one or more two-week treatment cycles, each two-week treatment cycle consisting of the administration, starting on the first day of each two-week treatment cycle of: a. 60-80 mg/m^2 of liposomal irinotecan composition comprising irinotecan liposomes, the irinotecan liposomes in the liposomal irinotecan composition having a diameter of approximately 80-140 nm and an irinotecan terminal elimination half-life in the patient of about 21 to 48 hours, wherein the irinotecan liposomes comprise irinotecan encapsulated in a unilamellar lipid bilayer vesicle composed of phosphatidylcholine, cholesterol,

and a polyethyleneglycol-derivatized phosphatidyl-ethanolamine; the irinotecan liposomes administered in combination with b. 200 mg/m^2 of the (l) form of leucovorin; and c. 2,400 mg/m^2 of 5-fluorouracil.

Claim 41 depends from claim 40, and thus incorporates the new limitation added to claim 40 by this amendment. The Office Action does not indicate how Chen anticipates independent claim 40. Applicants respectfully submit that Chen does not disclose the methods covered by either independent claim 40, nor the rejected claims depending therefrom. Reconsideration and withdrawal of this rejection are requested.

With entry of this Response, claim 44 reads:

A method of treating pancreatic cancer in a human patient who has previously been treated with gemcitabine, the method comprising intravenously administering to the patient in need thereof an antineoplastic therapy once every two weeks, the antineoplastic therapy consisting of: a. 60, 70 or 80 mg/m^2 of liposomal irinotecan composition comprising irinotecan liposomes, the irinotecan liposomes in the liposomal irinotecan composition having a diameter of about 80-140 nm and an irinotecan terminal elimination half-life in the patient of about 21 to 48 hours, wherein the irinotecan liposomes comprise irinotecan encapsulated in a unilamellar lipid bilayer vesicle composed of phosphatidylcholine, cholesterol, and a polyethyleneglycol-derivatized phosphatidyl-ethanolamine; and then administering b. 200 mg/m^2 of the (l) form of leucovorin over 30 minutes; and then administering c. 2,400 mg/m^2 of 5-fluorouracil over 46 hours; wherein the irinotecan is converted to SN-38 within the human patient and the AUC of the SN-38 increases less than proportionally with the dose of the liposomal irinotecan.

The Office Action does not indicate how Chen anticipates independent claim 44. Applicants respectfully submit that Chen does not disclose the methods covered by either independent claim 44, nor the rejected claims depending therefrom. Reconsideration and withdrawal of this rejection are requested.

The claimed methods include the steps of administering "60-80 mg/m^2," or "60, 70 or 80 mg/m^2" of liposomal irinotecan compositions comprising irinotecan liposomes. The liposomal irinotecan dose of 80 mg/m^2 is based on the amount of irinotecan in 80 mg/m^2 of CPT-11 irinotecan liposome trihydrate, and is equivalent to 70 mg/m^2 irinotecan free base. As explained in paragraph [0004] of the specification, CPT-11 irinotecan is marketed as CAMPTOSAR® (irinotecan hydrochloride injection), comprising irinotecan as irinotecan hydrochloride trihydrate (See CAMPTOSAR Prescribing Information dated May 14, 2010, attached as Tab 1). MM-398 is a liposomal form of CPT-11 (paragraph [0099] of the specification) approved by the FDA as the product ONIVYDE® (irinotecan liposome injection) "in combination with 5-fluorouracil and leucovorin for the treatment of patients with metastatic adenocarcinoma of the pancreas after disease progression following gemcitabine-based therapy" (Section 1 of the ONIVYDE Prescribing Information, attached as Tab 2).

In the present specification, "80 mg/m^2 of MM-3981iposomal irinotecan" refers to the hydrochloride trihydrate form of irinotecan, and is equivalent to the 70 mg/m^2 of the ONIVYDE dose recited in the ONIVYDE® Prescribing Information. For example, paragraph [0060] of the present specification refers to the Prescribing Information for Camptosar® (irinotecan hydrochloride) and discusses results in 66 patients who received single agent irinotecan (350 mg/m^2 once every-3-weeks). That 350 mg/m^2 dose is based on the hydrochloride trihydrate salt of irinotecan (Camptosar® Prescribing Information, page 11, Section 5.3 and page 24, Section 11)(attached in Tab 1). Furthermore, the results reported in Figure 5 of the present specification, for example, compare pharmacokinetic results for different doses of Camptosar® and irinotecan sucrose sulfate liposomal formulations and are reported on the basis of the hydrochloride trihydrate salt of irinotecan (See also paragraph [0106] of the present specification discussing the results reported in Figure 5).

In view of the above-mentioned amendment, applicant submits that Chen does not anticipate the present claims because Chen does not teach or suggest each and every element required by the claims. Accordingly, applicant respectfully requests reconsideration and withdrawal of the present 35 USC 102(b) rejection of the claims. Furthermore, Applicant has cancelled claims 30-32, 35, 36, 38, 39, 42, 43 and 45 rendering the rejection of these claims moot.

Double Patenting

The Examiner has provisionally rejected claims 28-45 on the ground of the judicially created doctrine of non-statutory double patenting as allegedly being unpatentable over claims 1-30 of co-pending Application No. 14/844,500, over claims 1-20 of copending Application No. 14/851,111 and over claims 1-12, 14, 15 and 20-32 of allowed (but not issued) Application No. 14/812,950 (Office Action at pages 7-8).

Terminal disclaimers in compliance with 37 C.F.R. 1.321(c) are being electronically filed herewith, along with the required fee of $160.00, rendering moot the basis for this rejection. Applicant respectfully requests reconsideration and withdrawal of this rejection.

CONCLUSION

Applicant has provided a full and complete response to the Office Action. The application is in condition for allowance. Applicant respectfully requests entry of this paper, favorable reconsideration and withdrawal of the rejections of the application, and prompt issuance of a Notice of Allowance.

Applicant believes that no further fees are required for the filing of this Response. However, if there are any additional charges or credits in direct relation to this filing please charge them to Deposit Account No.: 503145, referencing Attorney Docket No.: 239669-373010.

Respectfully submitted,

HONIGMAN MILLER SCHWARTZ AND COHN LLP

Dated: April 25, 2016

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HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use CAMPTOSAR safely and effectively. See full prescribing information for CAMPTOSAR.

CAMPTOSAR (Irinotecan) Injection, intravenous infusion
Initial U.S. Approval: 1996

WARNING: DIARRHEA and MYELOSUPPRESSION
See full prescribing information for complete boxed warning.

- **Early and late forms of diarrhea can occur. Early diarrhea may be accompanied by cholinergic symptoms which may be prevented or ameliorated by atropine. Late diarrhea can be life threatening and should be treated promptly with loperamide. Monitor patients with diarrhea and give fluid and electrolytes as needed. Institute antibiotic therapy if patients develop ileus, fever, or severe neutropenia. Interrupt CAMPTOSAR and reduce subsequent doses if severe diarrhea occurs.**
- **Severe myelosuppression may occur.**

----------INDICATIONS AND USAGE----------

CAMPTOSAR is a topoisomerase inhibitor indicated for:

- First-line therapy in combination with 5-fluorouracil and leucovorin for patients with metastatic carcinoma of the colon or rectum. (1)
- Patients with metastatic carcinoma of the colon or rectum whose disease has recurred or progressed following initial fluorouracil-based therapy. (1)

----------DOSAGE AND ADMINISTRATION----------

- Colorectal cancer combination regimen 1: CAMPTOSAR 125 mg/m^2 intravenous infusion over 90 minutes on days 1, 8,15, 22 with LV 20 mg/m^2 intravenous bolus infusion on days 1, 8, 15, 22 followed by 5-FU intravenous bolus infusion on days 1, 8, 15, 22 every 6 weeks. (2.1)
- Colorectal cancer combination regimen 2: CAMPTOSAR 180 mg/m^2 intravenous infusion over 90 minutes on days 1, 15, 29 with LV 200 mg/m^2 intravenous infusion over 2 hours on days 1, 2, 15, 16, 29, 20 followed by 5-FU 400 mg/m^2 intravenous bolus infusion on days 1, 2, 15, 16, 29, 30 and 5-FU 600 mg/m^2 intravenous infusion over 22 hours on days 1, 2, 15, 16, 29, 30. (2.1)
- Colorectal cancer single agent regimen 1: CAMPTOSAR 125 mg/m^2 intravenous infusion over 90 minutes on days 1, 8, 15, 22 then 2-week rest. (2.2)
- Colorectal cancer single agent regimen 2: CAMPTOSAR 350 mg/m^2 intravenous infusion over 90 minutes on day 1 every 3 weeks. (2.2)

----------DOSAGE FORMS AND STRENGTHS----------

CAMPTOSAR Injection is available in three single-dose sizes:

- 2 mL-fill vial containing 40 mg irinotecan hydrochloride injection
- 5 mL-fill vial containing 100 mg irinotecan hydrochloride injection
- 15 mL-fill vial containing 300 mg irinotecan hydrochloride injection

----------CONTRAINDICATIONS----------

- Hypersensitivity to CAMPTOSAR or its excipients (4)

----------WARNINGS AND PRECAUTIONS----------

- **Diarrhea and cholinergic reactions:** Early diarrhea (occurring during or shortly after infusion of CAMPTOSAR) is usually transient and may be accompanied by cholinergic symptoms. Consider prophylactic or therapeutic administration of 0.25 mg to 1 mg of intravenous or subcutaneous atropine (unless clinically contraindicated). Late diarrhea (generally occurring more than 24 hours after administration of CAMPTOSAR) can occur. Monitor and replace fluid and electrolytes. Treat with loperamide. Use antibiotic support for ileus and fever. Interrupt CAMPTOSAR and reduce subsequent doses if severe diarrhea occurs. (5.1)
- **Myelosuppression:** Manage promptly with antibiotic support. Interrupt CAMPTOSAR and reduce subsequent doses if necessary. (5.2)
- **Patients with Reduced UGT1A1 Activity:** Individuals who are homozygous for the UGT1A1*28 allele are at increased risk for neutropenia following initiation of CAMPTOSAR treatment. (5.3)
- **Hypersensitivity:** Hypersensitivity reactions including severe anaphylactic or anaphylactoid reactions have been observed. Discontinue CAMPTOSAR if this occurs. (5.4)
- **Renal Impairment/Renal Failure:** Rare cases of renal impairment and acute renal failure have been identified, usually in patients who became volume depleted from severe vomiting and/or diarrhea. (5.5)
- **Pulmonary Toxicity:** Interstitial Pulmonary Disease (IPD)-like events, including fatalities, have occurred. Interrupt for new or progressive dysnpnea, cough, and fever pending evaluation. If IPD diagnosed, discontinue and institute appropriate treatment as needed. (5.6)
- **Toxicity of the 5 Day Regimen:** CAMPTOSAR should not be used in combination with a regimen of 5-FU/LV administered for 4-5 consecutive days every 4 weeks outside of a clinical study. (5.7)
- **Pregnancy:** CAMPTOSAR can cause fetal harm when administered to a pregnant woman. (5.9)
- **Hepatic Impairment:** In clinical trials, CAMPTOSAR has not been administered to patients with serum bilirubin > 2.0 mg/dL, or transaminases > 3 times ULN if no liver metastases, or transaminases > 5 times ULN if liver metastases. With the weekly dosage schedule, patients with total bilirubin levels 1.0-2.0 mg/dL had greater likelihood of grade 3-4 neutropenia. (5.10)

----------ADVERSE REACTIONS----------

Common adverse reactions (≥30%) observed in combination therapy clinical studies are: nausea, vomiting, abdominal pain, diarrhea, constipation, anorexia, mucositis, neutropenia, leukopenia (including lymphocytopenia), anemia, thrombocytopenia, asthenia, pain, fever, infection, abnormal bilirubin, alopecia. (6.1)

Common adverse reactions (≥30%) observed in single agent therapy clinical studies are: nausea, vomiting, abdominal pain, diarrhea, constipation, anorexia, neutropenia, leukopenia (including lymphocytopenia), anemia, asthenia, fever, body weight decreasing, alopecia. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Pfizer Inc at 1-800-438-1985 or *www.pfizer.com* or FDA at 1-800-FDA-1088 or *www.fda.gov/medwatch*.

----------DRUG INTERACTIONS----------

- Strong CYP3A4 Inducers: Do not administer for at least 2 weeks prior to initiation of irinotecan therapy. (7.2)
- Strong CYP3A4 Inhibitors: Discontinue at least 1 week prior to starting irinotecan therapy and do not use during irinotecan therapy. (7.3)

----------USE IN SPECIFIC POPULATIONS----------

- **Nursing Mothers:** Discontinue nursing when receiving therapy with CAMPTOSAR. (8.3)
- **Geriatric Use:** Closely monitor patients greater than 65 years of age because of a greater risk of early and late diarrhea in this population. (8.5)
- **Patients with Renal Impairment**: Use caution and do not use in patients on dialysis. (8.6)
- **Patients with Hepatic Impairment**: Use caution. (2.1, 5.10, 8.7, 12.3)

See 17 for PATIENT COUNSELING INFORMATION.

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FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

WARNING: DIARRHEA AND MYELOSUPPRESSION

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- **Severe myelosuppression may occur.**

1 INDICATIONS AND USAGE

- CAMPTOSAR Injection is indicated as a component of first-line therapy in combination with 5-fluorouracil (5-FU) and leucovorin (LV) for patients with metastatic carcinoma of the colon or rectum.

- CAMPTOSAR is indicated for patients with metastatic carcinoma of the colon or rectum whose disease has recurred or progressed following initial fluorouracil-based therapy.

2 DOSAGE AND ADMINISTRATION

2.1 Colorectal Cancer Combination Regimens 1 and 2

Administer CAMPTOSAR as a 90-minute intravenous infusion followed by LV and 5-FU. The currently recommended regimens are shown in Table 1.

A reduction in the starting dose by one dose level of CAMPTOSAR may be considered for patients with any of the following conditions: prior pelvic/abdominal radiotherapy, performance status of 2, or increased bilirubin levels. Dosing for patients with bilirubin >2 mg/dL cannot be recommended because there is insufficient information to recommend a dose in these patients.

Table 1. Combination-Agent Dosage Regimens and Dose Modifications[a]

Regimen 1 6-wk cycle with bolus 5-FU/LV (next cycle begins on day 43)	CAMPTOSAR LV 5-FU	125 mg/m^2 intravenous infusion over 90 minutes, days 1,8,15,22 20 mg/m^2 intravenous injection bolus, days 1,8,15,22 500 mg/m^2 intravenous injection bolus, days 1,8,15,22		
		Starting Dose & Modified Dose Levels (mg/m^2)		
		Starting Dose	Dose Level -1	Dose Level -2
	CAMPTOSAR	125	100	75
	LV	20	20	20
	5-FU	500	400	300
Regimen 2 6-wk cycle with infusional 5-FU/LV (next cycle begins on day 43)	CAMPTOSAR LV 5-FU Bolus 5-FU Infusion[b]	180 mg/m^2 intravenous infusion over 90 minutes, days 1,15,29 200 mg/m^2 intravenous infusion over 2 hours, days 1,2,15,16,29,30 400 mg/m^2 intravenous injection bolus, days 1,2,15,16,29,30 600 mg/m^2 intravenous infusion over 22 hours, days 1,2,15,16,29,30		
		Starting Dose & Modified Dose Levels (mg/m^2)		
		Starting Dose	Dose Level -1	Dose Level -2
	CAMPTOSAR	180	150	120
	LV	200	200	200
	5-FU Bolus	400	320	240
	5-FU Infusion[b]	600	480	360

[a]Dose reductions beyond Dose Level –2 by decrements of ≈ 20% may be warranted for patients continuing to experience toxicity. Provided intolerable toxicity does not develop, treatment with additional cycles may be continued indefinitely as long as patients continue to experience clinical benefit.
[b]Infusion follows bolus administration.

Dosing for patients with bilirubin >2 mg/dL cannot be recommended because there is insufficient information to recommend a dose in these patients *[see Warnings and Precautions (5.10), Use in Specific Populations (8.7) and Clinical Pharmacology (12.3)]*.

Dose Modifications

Based on recommended dose levels described in Table 1, Combination Regimens of CAMPTOSAR and Dose Modifications, subsequent doses should be adjusted as suggested in Table 2, Recommended Dose Modifications for Combination Regimens. All dose modifications should be based on the worst preceding toxicity.