INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/057542

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/17 A61K31/4745 A61K31/505 A61K31/519 A61P35/00
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 A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Sanofi-Regeneron: "Aflibercept Versus Placebo in Combination With Irinotecan and 5-FU in theTreatment of Patients With Metastatic Colorectal Cancer After Failure of an Oxaliplatin Based Regimen", ClinicalTrials.gov , 17 February 2011 (2011-02-17), XP002677282, Retrieved from the Internet: URL:http://clinicaltrials.gov/archive/NCT00561470/2011_02_17 [retrieved on 2012-06-05] the whole document ----- -/--	26-32

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

"E" earlier application or patent but published on or after the international filing date

"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
6 June 2012	19/06/2012

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HURWITZ H ET AL: "BAVACIZUMAB PLUS IRINOTECAN, FLUOROURACIL, AND LEUCOVORIN FOR METASTATIC COLORECTAL CANCER", NEW ENGLAND JOURNAL OF MEDICINE, MASSACHUSETTS MEDICAL SOCIETY, BOSTON, MA, US, vol. 350, no. 23, 3 June 2004 (2004-06-03) , pages 2335-2342, XP009038752, ISSN: 1533-4406, DOI: 10.1056/NEJMOA032691 see abstract and Table 1	31
X	WO 2009/024667 A2 (AVENTIS PHARMA SA [FR]; BISSERY MARIE-CHRISTINE [FR]; CHIRON-BLONDEL M) 26 February 2009 (2009-02-26) see pages 2-3, page 6 l.13-16, page 7 last paragraph	26-31
X	WO 2006/059012 A1 (AVENTIS PHARMA SA [FR]) 8 June 2006 (2006-06-08) see claims, page 5 l.17-20 and example	26-31

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/057542

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009024667 A2	26-02-2009	AR 067420 A1	07-10-2009
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		AU 2008290442 A1	26-02-2009
		CA 2693152 A1	26-02-2009
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		DK 2173349 T3	27-06-2011
		EP 2173349 A2	14-04-2010
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		FR 2918279 A1	09-01-2009
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		FR 2878749 A1	09-06-2006
		HR 20090336 T1	31-07-2009
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		KR 20070091130 A	07-09-2007
		PT 1824504 E	25-06-2009
		RU 2384344 C2	20-03-2010
		SI 1824504 T1	31-08-2009
		US 2006178305 A1	10-08-2006
		US 2009285841 A1	19-11-2009
		WO 2006059012 A1	08-06-2006

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use ABRAXANE safely and effectively. See full prescribing information for ABRAXANE

ABRAXANE® for Injectable Suspension (paclitaxel protein-bound particles for injectable suspension) (albumin-bound)
Initial U.S. Approval: 2005

WARNING: NEUTROPENIA
See full prescribing information for complete boxed warning.

- **ABRAXANE therapy should not be administered to patients with baseline neutrophil counts of less than 1,500 cells/mm^3 (4).**
- **It is recommended that frequent peripheral blood cell counts be performed to monitor the occurrence of bone marrow suppression. (4, 5.1, 6.1)**

DO NOT SUBSTITUTE FOR OR WITH OTHER PACLITAXEL FORMULATIONS

---------- RECENT MAJOR CHANGES ----------

---------- INDICATIONS AND USAGE ----------
ABRAXANE is a microtubule inhibitor indicated for the treatment of breast cancer after failure of combination chemotherapy for metastatic disease or relapse within 6 months of adjuvant chemotherapy. Prior therapy should have included an anthracycline unless clinically contraindicated.

---------- DOSAGE AND ADMINISTRATION ----------
- Recommended dosage: 260 mg/m^2 IV over 30 min every 3 weeks (2.1)
- No adjustment is necessary for patients with mild hepatic impairment. Patients should not receive ABRAXANE if AST > 10 x ULN or bilirubin > 5.0 x ULN. Reduce starting dose in patients with moderate to severe hepatic impairment. (2.2)
- In case of severe neutropenia or severe sensory neuropathy reduce dose to 220 mg/m^2 for subsequent courses. In case of recurrence, further reduce dose to 180 mg/m^2. For grade 3 sensory neuropathy hold treatment until resolution to grade 1 or 2, followed by a dose reduction for all subsequent courses. (2.3)
- Use caution when handling cytotoxic drugs. Closely monitor the infusion site for extravasation and infiltration. No premedication is required prior to administration. (2.4)

---------- DOSAGE FORMS AND STRENGTHS ----------
- Single use vial containing 100 mg of paclitaxel (3)

---------- CONTRAINDICATIONS ----------
- Neutrophil counts of < 1,500 cells/mm^3. (4)
- Severe hypersensitivity reaction to ABRAXANE (4)

---------- WARNINGS AND PRECAUTIONS ----------
- ABRAXANE causes myelosuppression. Monitor CBC and withhold and/or reduce the dose as needed. (5.1)
- Sensory neuropathy occurs frequently and may require dose reduction or treatment interruption. (5.2)
- Exposure and toxicity of paclitaxel can be increased in patients with hepatic impairment; therefore administer with caution. (5.3)
- ABRAXANE contains albumin derived from human blood which has a theoretical risk of viral transmission. (5.4)
- Fetal harm may occur when administered to a pregnant woman. Women of childbearing potential should avoid becoming pregnant while receiving ABRAXANE. (5.5)
- Men should not father a child while on ABRAXANE. (5.6)

---------- ADVERSE REACTIONS ----------
The most common adverse reactions (≥ 20%) are alopecia, neutropenia, sensory neuropathy, abnormal ECG, fatigue/asthenia, myalgia/arthralgia, AST elevation, alkaline phosphatase elevation, anemia, nausea, infections, and diarrhea. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Celgene Corporation at 1-888-423-5436 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

---------- DRUG INTERACTIONS ----------
- Use caution when concomitantly administering ABRAXANE with inhibitors or inducers of either CYP2C8 or CYP3A4. (7)

See 17 for PATIENT COUNSELING INFORMATION and see FDA-approved patient labeling.

Revised: 12/2011

FULL PRESCRIBING INFORMATION: CONTENTS*

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FULL PRESCRIBING INFORMATION
ABRAXANE® for Injectable Suspension (paclitaxel protein-bound particles for injectable suspension) (albumin-bound)

WARNING: NEUTROPENIA

- **ABRAXANE therapy should not be administered to patients with metastatic breast cancer who have baseline neutrophil counts of less than 1,500 cells/mm^3. In order to monitor the occurrence of bone marrow suppression, primarily neutropenia, which may be severe and result in infection, it is recommended that frequent peripheral blood cell counts be performed on all patients receiving ABRAXANE** *[see Contraindications (4), Warnings and Precautions (5.1) and Adverse Reactions (6.1)].*
- **Note: An albumin form of paclitaxel may substantially affect a drug's functional properties relative to those of drug in solution. DO NOT SUBSTITUTE FOR OR WITH OTHER PACLITAXEL FORMULATIONS.**

1 INDICATIONS AND USAGE

ABRAXANE for Injectable Suspension (paclitaxel protein-bound particles for injectable suspension) is indicated for the treatment of breast cancer after failure of combination chemotherapy for metastatic disease or relapse within 6 months of adjuvant chemotherapy. Prior therapy should have included an anthracycline unless clinically contraindicated.

2 DOSAGE AND ADMINISTRATION

2.1 General

After failure of combination chemotherapy for metastatic breast cancer or relapse within 6 months of adjuvant chemotherapy, the recommended regimen for ABRAXANE is 260 mg/m^2 administered intravenously over 30 minutes every 3 weeks.

2.2 Dosage in Patients with Hepatic Impairment

No dose adjustment is necessary for patients with mild hepatic impairment. Patients with moderate and severe hepatic impairment treated with ABRAXANE may be at increased risk of toxicities known to paclitaxel. Patients should not receive ABRAXANE if AST > 10 x ULN or bilirubin > 5.0 x ULN. Recommendations for dosage adjustment for the first course of therapy are shown in Table 1. The dose of ABRAXANE can be increased up to 200 mg/m^2 in patients with severe hepatic impairment in subsequent cycles based on individual tolerance. Patients should be monitored closely *[see Clinical Pharmacology (12.3) and Warnings and Precautions (5.3) and Use in Specific Populations (8.6)].*

Table 1: Recommendations for Starting Dose in Patients with Hepatic Impairment

	SGOT (AST) Levels		Bilirubin Levels	ABRAXANE [a]
Mild	< 10 x ULN		> ULN to ≤ 1.25 x ULN	260 mg/m^2
Moderate	< 10 x ULN	AND	1.26 to 2.0 x ULN	200 mg/m^2
Severe	< 10 x ULN		2.01 to 5.0 x ULN	130 mg/m^2 [b]
	> 10 x ULN	OR	> 5.0 x ULN	not eligible

[a] Dosage recommendations are for the first course of therapy. The need for further dose adjustments in subsequent courses should be based on individual tolerance.
[b] A dose increase to 200 mg/m^2 in subsequent courses should be considered based on individual tolerance.

2.3 Dose Reduction: in Case of Severe Neutropenia or Severe Sensory Neuropathy

Patients who experience severe neutropenia (neutrophil <500 cells/mm^3 for a week or longer) or severe sensory neuropathy during ABRAXANE therapy should have dosage reduced to 220 mg/m^2 for subsequent courses of ABRAXANE. For recurrence of severe neutropenia or severe sensory neuropathy, additional dose reduction should be made to 180 mg/m^2. For grade 3 sensory neuropathy hold treatment until resolution to grade 1 or 2, followed by a dose reduction for all subsequent courses of ABRAXANE *[see Contraindications (4), Warnings and Precautions (5.1 and 5.2) and Adverse Reactions (6.1)].*

2.4 Preparation and Administration Precautions

ABRAXANE is a cytotoxic drug and, as with other potentially toxic paclitaxel compounds, caution should be exercised in handling ABRAXANE. The use of gloves is recommended. If ABRAXANE (lyophilized cake or reconstituted suspension) contacts the skin, wash the skin immediately and thoroughly with soap and water. Following topical exposure to paclitaxel, events may include tingling, burning and redness. If ABRAXANE contacts mucous membranes, the membranes should be flushed thoroughly with water.

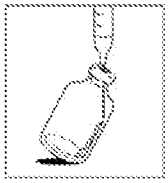
Given the possibility of extravasation, it is advisable to closely monitor the infusion site for possible infiltration during drug administration. Limiting the infusion of ABRAXANE to 30 minutes, as directed, reduces the likelihood of infusion-related reactions *[see Adverse Reactions (6.2)].*

No premedication to prevent hypersensitivity reactions is required prior to administration of ABRAXANE.

2.5 Preparation for Intravenous Administration

ABRAXANE is supplied as a sterile lyophilized powder for reconstitution before use. **AVOID ERRORS, READ ENTIRE PREPARATION INSTRUCTIONS PRIOR TO RECONSTITUTION.**

1. Aseptically, reconstitute each vial by injecting 20 mL of 0.9% Sodium Chloride Injection, USP.
2. Slowly inject the 20 mL of 0.9% Sodium Chloride Injection, USP, over a minimum of 1 minute, using the sterile syringe to direct the solution flow onto the INSIDE WALL OF THE VIAL.



3. DO NOT INJECT the 0.9% Sodium Chloride Injection, USP, directly onto the lyophilized cake as this will result in foaming.
4. Once the injection is complete, allow the vial to sit for a minimum of 5 minutes to ensure proper wetting of the lyophilized cake/powder.
5. Gently swirl and/or invert the vial slowly for at least 2 minutes until complete dissolution of any cake/powder occurs. Avoid generation of foam.
6. If foaming or clumping occurs, stand solution for at least 15 minutes until foam subsides.

Each mL of the reconstituted formulation will contain 5 mg/mL paclitaxel.

Calculate the exact total dosing volume of 5 mg/mL suspension required for the patient: Dosing volume (mL) = Total dose (mg)/5 (mg/mL)

The reconstituted suspension should be milky and homogenous without visible particulates. If particulates or settling are visible, the vial should be **gently** inverted again to ensure complete resuspension prior to use. Discard the reconstituted suspension if precipitates are observed. Discard any unused portion.

Inject the appropriate amount of reconstituted ABRAXANE into an empty, sterile IV bag [plasticized polyvinyl chloride (PVC) containers, PVC or non-PVC type IV bag]. The use of specialized DEHP-free solution containers or administration sets is not necessary to prepare or administer ABRAXANE infusions. The use of an in line filter is not recommended.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

2.6 Stability

Unopened vials of ABRAXANE are stable until the date indicated on the package when stored between 20°C to 25°C (68°F to 77°F) in the original package. Neither freezing nor refrigeration adversely affects the stability of the product.

Stability of Reconstituted Suspension in the Vial

Reconstituted ABRAXANE in the vial should be used immediately, but may be refrigerated at 2°C to 8°C (36°F to 46°F) for a maximum of 8 hours if necessary. If not used immediately, each vial of reconstituted suspension should be replaced in the original carton to protect it from bright light. Discard any unused portion.

Stability of Reconstituted Suspension in the Infusion Bag

The suspension for infusion when prepared as recommended in an infusion bag should be used immediately but may be stored at ambient temperature (approximately 25°C) and lighting conditions for up to 4 hours. Discard any unused portion.

3 DOSAGE FORMS AND STRENGTHS

Single use vials containing 100 mg of paclitaxel.

4 CONTRAINDICATIONS

ABRAXANE should not be used in patients who have baseline neutrophil counts of < 1,500 cells/mm^3. Patients who experience a severe hypersensitivity reaction to ABRAXANE should not be rechallenged with the drug.

5 WARNINGS AND PRECAUTIONS

5.1 Hematologic Effects

Bone marrow suppression (primarily neutropenia) is dose dependent and a dose limiting toxicity. ABRAXANE should not be administered to patients with baseline neutrophil counts of less than 1,500 cells/mm^3. In order to monitor the occurrence of myelotoxicity, perform frequent peripheral blood cell counts. Retreat with subsequent cycles of ABRAXANE after neutrophils recover to a level >1,500 cells/mm^3 and platelets recover to a level >100,000 cells/mm^3. In the case of severe neutropenia (<500 cells/mm^3

for seven days or more) during a course of ABRAXANE therapy, dose reduce for subsequent courses of therapy. *[see Dosage and Administration (2.3)]*.

5.2 Nervous System
Sensory neuropathy occurs frequently with ABRAXANE. The occurrence of grade 1 or 2 sensory neuropathy does not generally require dose modification. If grade 3 sensory neuropathy develops, treatment should be withheld until resolution to grade 1 or 2 followed by a dose reduction for all subsequent courses of ABRAXANE *[see Dosage and Administration (2.3)]*.

5.3 Hepatic Impairment
Because the exposure and toxicity of paclitaxel can be increased with hepatic impairment, administration of ABRAXANE in patients with hepatic impairment should be performed with caution. The starting dose should be reduced for patients with moderate and severe hepatic impairment *[see Dosage and Administration (2.2), Use in Specific Populations (8.6) and Clinical Pharmacology (12.3)]*.

5.4 Albumin (Human)
ABRAXANE contains albumin (human), a derivative of human blood. Based on effective donor screening and product manufacturing processes, it carries a remote risk for transmission of viral diseases. A theoretical risk for transmission of Creutzfeldt-Jakob Disease (CJD) also is considered extremely remote. No cases of transmission of viral diseases or CJD have ever been identified for albumin.

5.5 Use in Pregnancy
ABRAXANE can cause fetal harm when administered to a pregnant woman. Administration of paclitaxel protein-bound particles to rats during pregnancy at doses lower than the maximum recommended human dose, based on body surface area, caused embryo-fetal toxicities, including intrauterine mortality, increased resorptions, reduced numbers of live fetuses, and malformations.

There are no adequate and well-controlled studies in pregnant women receiving ABRAXANE. If this drug is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant while receiving ABRAXANE *[see Use in Specific Populations (8.1)]*.

5.6 Use in Men
Men should be advised not to father a child while receiving ABRAXANE. *[see Nonclinical Toxicology (13.1)]*.

6 ADVERSE REACTIONS
Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The most common adverse reactions (≥ 20%) are alopecia, neutropenia, sensory neuropathy, abnormal ECG, fatigue/asthenia, myalgia/arthralgia, AST elevation, alkaline phosphatase elevation, anemia, nausea, infections, diarrhea.

6.1 Clinical Trials Experience
The following table shows the frequency of important adverse events in the randomized comparative trial for the patients who received either single-agent ABRAXANE or paclitaxel injection for the treatment of metastatic breast cancer.

Table 2: Frequency[a] of Important Treatment Emergent Adverse Events in the Randomized Study on an Every-3-Weeks Schedule

	Percent of Patients	
	ABRAXANE® 260 mg/m^2 over 30 min (n=229)	**Paclitaxel Injection 175 mg/m^2 over 3 h[b] (n=225)**
Bone Marrow		
Neutropenia		
< 2.0 x 10^9/L	80	82
< 0.5 x 10^9/L	9	22
Thrombocytopenia		
< 100 x 10^9/L	2	3
< 50 x 10^9/L	<1	<1
Anemia		
< 11 g/dL	33	25
< 8 g/dL	1	<1
Infections	24	20
Febrile Neutropenia	2	1
Bleeding	2	2
Hypersensitivity Reaction[c]		
All	4	12
Severe[d]	0	2

	Percent of Patients	
	ABRAXANE® 260 mg/m^2 over 30 min (n=229)	**Paclitaxel Injection 175 mg/m^2 over 3 h[b] (n=225)**
Cardiovascular		
Vital Sign Changes During Administration		
Bradycardia	<1	<1
Hypotension	5	5
Severe Cardiovascular Events[d]	3	4
Abnormal ECG		
All patients	60	52
Patients with Normal Baseline	35	30
Respiratory		
Cough	7	6
Dyspnea	12	9
Sensory Neuropathy		
Any Symptoms	71	56
Severe Symptoms[d]	10	2
Myalgia / Arthralgia		
Any Symptoms	44	49
Severe Symptoms[d]	8	4
Asthenia		
Any Symptoms	47	39
Severe Symptoms[d]	8	3
Fluid Retention/Edema		
Any Symptoms	10	8
Severe Symptoms[d]	0	<1
Gastrointestinal		
Nausea		
Any symptoms	30	22
Severe symptoms[d]	3	<1
Vomiting		
Any symptoms	18	10
Severe Symptoms[d]	4	1
Diarrhea		
Any Symptoms	27	15
Severe Symptoms[d]	<1	1
Mucositis		
Any Symptoms	7	6
Severe Symptoms[d]	<1	0
Alopecia	90	94
Hepatic (Patients with Normal Baseline)		
Bilirubin Elevations	7	7
Alkaline Phosphatase Elevations	36	31
AST (SGOT) Elevations	39	32
Injection Site Reaction	<1	1

[a] Based on worst grade by NCI Common Terminology Criteria for Adverse Events (CTCAE) version 2.
[b] Paclitaxel injection pts received premedication.
[c] Includes treatment-related events related to hypersensitivity (e.g., flushing, dyspnea, chest pain, hypotension) that began on a day of dosing.
[d] Severe events are defined as at least grade 3 toxicity.

Adverse Event Experiences by Body System

Hematologic Disorders
Neutropenia was dose dependent and reversible. Among patients with metastatic breast cancer in the randomized trial, neutrophil counts declined below 500 cells/mm^3 (Grade 4) in 9% of the patients treated with a dose of 260 mg/m^2 compared to 22% in patients receiving paclitaxel injection at a dose of 175 mg/m^2. Pancytopenia has been observed in clinical trials.

Infections
Infectious episodes were reported in 24% of the patients treated with ABRAXANE. Oral candidiasis, respiratory tract infections and pneumonia were the most frequently reported infectious complications.

Hypersensitivity Reactions (HSRs)
Grade 1 or 2 HSRs occurred on the day of ABRAXANE administration and consisted of dyspnea (1%) and flushing, hypotension, chest pain, and arrhythmia (all <1%). The use of ABRAXANE in patients previously exhibiting hypersensitivity to paclitaxel injection or human albumin has not been studied.

Cardiovascular
Hypotension, during the 30-minute infusion, occurred in 5% of patients. Bradycardia, during the 30-minute infusion, occurred in <1% of patients. These vital sign changes most often caused no symptoms and required neither specific therapy nor treatment discontinuation.

Severe cardiovascular events possibly related to single-agent ABRAXANE occurred in approximately 3% of patients.. These events included cardiac ischemia/infarction, chest pain, cardiac arrest, supraventricular tachycardia, edema, thrombosis, pulmonary thromboembolism, pulmonary emboli, and hypertension. Cases of cerebrovascular attacks (strokes) and transient ischemic attacks have been reported.

Electrocardiogram (ECG) abnormalities were common among patients at baseline. ECG abnormalities on study did not usually result in symptoms, were not dose-limiting, and required no intervention. ECG abnormalities were noted in 60% of patients. Among patients with a normal ECG prior to study entry, 35% of all patients developed an abnormal tracing while on study. The most frequently reported ECG modifications were non-specific repolarization abnormalities, sinus bradycardia, and sinus tachycardia.

Respiratory
Dyspnea (12%), cough (7%), and pneumothorax (<1%) were reported after treatment with ABRAXANE.

Neurologic
The frequency and severity of sensory neuropathy increased with cumulative dose. Sensory neuropathy was the cause of ABRAXANE discontinuation in 7/229 (3%) patients. Twenty-four patients (10%) treated with ABRAXANE developed Grade 3 peripheral neuropathy; of these patients, 14 had documented improvement after a median of 22 days; 10 patients resumed treatment at a reduced dose of ABRAXANE and 2 discontinued due to peripheral neuropathy. Of the 10 patients without documented improvement, 4 discontinued the study due to peripheral neuropathy.

No grade 4 sensory neuropathies were reported. Only one incident of motor neuropathy (grade 2) was observed in either arm of the controlled trial.

Vision Disorders
Ocular/visual disturbances occurred in 13% of all patients (n=366) treated with ABRAXANE and 1% were severe. The severe cases (keratitis and blurred vision) were reported in patients who received higher doses than those recommended (300 or 375 mg/m^2). These effects generally have been reversible.

Arthralgia/Myalgia
The symptoms were usually transient, occurred two or three days after ABRAXANE administration, and resolved within a few days.

Hepatic
Grade 3 or 4 elevations in GGT were reported for 14% of patients treated with ABRAXANE and 10% of patients treated with paclitaxel injection in the randomized trial.

Renal
Overall 11% of patients experienced creatinine elevation, 1% severe. No discontinuations, dose reductions, or dose delays were caused by renal toxicities.

Other Clinical Events
Nail changes (changes in pigmentation or discoloration of nail bed) have been reported. Edema occurred in 10% of patients; no patients had severe edema. Dehydration and pyrexia were also reported.

6.2 Post-Marketing Experience with ABRAXANE and other Paclitaxel Formulations
Unless otherwise noted, the following discussion refers to the adverse reactions that have been identified during post-approval use of ABRAXANE. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure. In some instances, severe events observed with paclitaxel injection may be expected to occur with ABRAXANE.

Hypersensitivity Reactions
Severe hypersensitivity reactions have been reported with ABRAXANE. The use of ABRAXANE in patients previously exhibiting hypersensitivity to paclitaxel injection or human albumin has not been studied.

Cardiovascular
There have been reports of congestive heart failure and left ventricular dysfunction with ABRAXANE. Most of the individuals were previously exposed to cardiotoxic drugs, such as anthracyclines, or had underlying cardiac history.

Respiratory
There have been reports of interstitial pneumonia and pulmonary embolism in patients receiving ABRAXANE and reports of radiation pneumonitis in patients receiving concurrent radiotherapy. Reports of lung fibrosis have been received as part of the

continuing surveillance of paclitaxel injection safety and may also be observed with ABRAXANE.

Neurologic
Cranial nerve palsies and vocal cord paresis have been reported as has autonomic neuropathy resulting in paralytic ileus.

Vision Disorders
Reports in the literature of abnormal visual evoked potentials in patients treated with paclitaxel injection suggest persistent optic nerve damage. These may also be observed with ABRAXANE.

Hepatic
Reports of hepatic necrosis and hepatic encephalopathy leading to death have been received as part of the continuing surveillance of paclitaxel injection safety and may occur following ABRAXANE treatment.

Gastrointestinal (GI)
There have been reports of intestinal obstruction, intestinal perforation, pancreatitis, and ischemic colitis following ABRAXANE treatment. There have been reports of neutropenic enterocolitis (typhlitis), despite the coadministration of G-CSF, occurring in patients treated with paclitaxel injection alone and in combination with other chemotherapeutic agents.

Injection Site Reaction
There have been reports of extravasation of ABRAXANE. Given the possibility of extravasation, it is advisable to monitor closely the ABRAXANE infusion site for possible infiltration during drug administration.

Severe events such as phlebitis, cellulitis, induration, necrosis, and fibrosis have been reported as part of the continuing surveillance of paclitaxel injection safety. In some cases the onset of the injection site reaction in paclitaxel injection patients either occurred during a prolonged infusion or was delayed by a week to ten days. Recurrence of skin reactions at a site of previous extravasation following administration of paclitaxel injection at a different site, i.e., "recall", has been reported.

Other Clinical Events
Skin reactions including generalized or maculo-papular rash, erythema, and pruritus have been observed with ABRAXANE. There have been case reports of photosensitivity reactions, radiation recall phenomenon, and in some patients previously exposed to capecitabine, reports of palmar-plantar erythrodysesthesia. Stevens-Johnson syndrome and toxic epidermal necrolysis have been reported.

There have been reports of conjunctivitis, cellulitis, and increased lacrimation with paclitaxel injection.

6.3 Accidental Exposure
No reports of accidental exposure to ABRAXANE have been received. However, upon inhalation of paclitaxel, dyspnea, chest pain, burning eyes, sore throat, and nausea have been reported. Following topical exposure, events have included tingling, burning, and redness.

7 DRUG INTERACTIONS
No drug interaction studies have been conducted with ABRAXANE.

The metabolism of paclitaxel is catalyzed by CYP2C8 and CYP3A4. In the absence of formal clinical drug interaction studies, caution should be exercised when administering ABRAXANE concomitantly with medicines known to inhibit (e.g. ketoconazole and other imidazole antifungals, erythromycin, fluoxetine, gemfibrozil, cimetidine, ritonavir, saquinavir, indinavir, and nelfinavir) or induce (e.g. rifampicin, carbamazepine, phenytoin, efavirenz, and nevirapine) either CYP2C8 or CYP3A4.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy
Pregnancy Category D *[see Warnings and Precautions (5.5)]*.

There are no adequate and well-controlled studies in pregnant women using ABRAXANE. Based on its mechanism of action and findings in animals, ABRAXANE can cause fetal harm when administered to a pregnant woman. If this drug is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant while receiving ABRAXANE.

Administration of paclitaxel protein-bound particles to rats during pregnancy, on gestation days 7 to 17 at doses of 6 mg/m^2 (approximately 2% of the daily maximum recommended human dose on a mg/m^2 basis) caused embryofetal toxicities, as indicated by intrauterine mortality, increased resorptions (up to 5-fold), reduced numbers of litters and live fetuses, reduction in fetal body weight and increase in fetal anomalies. Fetal anomalies included soft tissue and skeletal malformations, such as eye bulge, folded retina, microphthalmia, and dilation of brain ventricles. A lower incidence of soft tissue and skeletal malformations were also exhibited at 3 mg/m^2 (approximately 1% of the daily maximum recommended human dose on a mg/m^2 basis).

8.3 Nursing Mothers
It is not known whether paclitaxel is excreted in human milk. Paclitaxel and/or its metabolites were excreted into the milk of lactating rats. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants, a decision should be made to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

8.4 Pediatric Use
The safety and effectiveness of ABRAXANE in pediatric patients have not been evaluated.

8.5 Geriatric Use
Of the 229 patients in the randomized study who received ABRAXANE, 13% were at least 65 years of age and < 2% were 75 years or older. No toxicities occurred notably more frequently among patients who received ABRAXANE.

8.6 Patients with Hepatic Impairment
Because the exposure and toxicity of paclitaxel can be increased in patients with hepatic impairment, the administration of ABRAXANE should be performed with caution in patients with hepatic impairment *[see Dosage and Administration (2.2), Warnings and Precautions (5.3) and Clinical Pharmacology (12.3)]*.

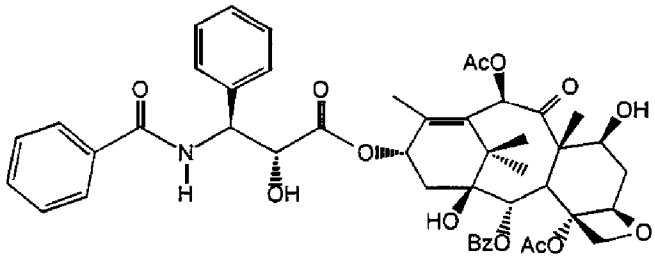
8.7 Patients with Renal Impairment
The use of ABRAXANE has not been studied in patients with renal impairment. Patients were excluded for baseline serum bilirubin >1.5 mg/dL or baseline serum creatinine >2 mg/dL.

10 OVERDOSAGE
There is no known antidote for ABRAXANE overdosage. The primary anticipated complications of overdosage would consist of bone marrow suppression, sensory neurotoxicity, and mucositis.

11 DESCRIPTION
ABRAXANE, a microtubule inhibitor, is an albumin-bound form of paclitaxel with a mean particle size of approximately 130 nanometers. Paclitaxel exists in the particles in a non-crystalline, amorphous state. ABRAXANE is supplied as a white to yellow, sterile, lyophilized powder for reconstitution with 20 mL of 0.9% Sodium Chloride Injection, USP prior to intravenous infusion. Each single-use vial contains 100 mg of paclitaxel (bound to human albumin) and approximately 900 mg of human albumin (containing sodium caprylate and sodium acetyltryptophanate). Each milliliter (mL) of reconstituted suspension contains 5 mg paclitaxel. ABRAXANE is free of solvents.

The active agent in ABRAXANE is paclitaxel. The chemical name for paclitaxel is 5β,20-Epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine.

Paclitaxel has the following structural formula:



Paclitaxel is a white to off-white crystalline powder with the empirical formula $C_{47}H_{51}NO_{14}$ and a molecular weight of 853.91. It is highly lipophilic, insoluble in water, and melts at approximately 216°C to 217°C.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action
ABRAXANE is a microtubule inhibitor that promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. This stability results in the inhibition of the normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic cellular functions. Paclitaxel induces abnormal arrays or "bundles" of microtubules throughout the cell cycle and multiple asters of microtubules during mitosis.

12.3 Pharmacokinetics

Absorption

The pharmacokinetics of total paclitaxel following 30 and 180-minute infusions of ABRAXANE at dose levels of 80 to 375 mg/m^2 were determined in clinical studies. Dose levels of mg/m^2 refer to mg of paclitaxel in ABRAXANE. Following intravenous administration of ABRAXANE, paclitaxel plasma concentrations declined in a biphasic manner, the initial rapid decline representing distribution to the peripheral compartment and the slower second phase representing drug elimination. The terminal half-life was about 27 hours.

The drug exposure (AUCs) was dose proportional over 80 to 375 mg/m^2 and the pharmacokinetics of paclitaxel for ABRAXANE were independent of the duration of administration. At the recommended ABRAXANE clinical dose, 260 mg/m^2, the mean maximum concentration of paclitaxel, which occurred at the end of the infusion, was 18,741 ng/mL. The mean total clearance was 15 L/hr/m^2. The mean volume of distribution was 632 L/m^2; the large volume of distribution indicates extensive extravascular distribution and/or tissue binding of paclitaxel.

The pharmacokinetic data of 260 mg/m^2 ABRAXANE administered over 30 minutes was compared to the pharmacokinetics of 175 mg/m^2 paclitaxel injection over 3 hours. The clearance of ABRAXANE was larger (43%) than for the clearance of paclitaxel injection and the volume of distribution of ABRAXANE was also higher (53%). Differences in C_{max} and C_{max} corrected for dose reflected differences in total dose and rate of infusion. There were no differences in terminal half-lives.

Distribution

In vitro studies of binding to human serum proteins, using paclitaxel concentrations ranging from 0.1 to 50 µg/mL, indicate that between 89% to 98% of drug is bound; the presence of cimetidine, ranitidine, dexamethasone, or diphenhydramine did not affect protein binding of paclitaxel.

Metabolism

In vitro studies with human liver microsomes and tissue slices showed that paclitaxel was metabolized primarily to 6α-hydroxypaclitaxel by CYP2C8; and to two minor metabolites, 3'-*p*-hydroxypaclitaxel and 6α, 3'-*p*-dihydroxypaclitaxel, by CYP3A4. *In vitro*, the metabolism of paclitaxel to 6α-hydroxypaclitaxel was inhibited by a number of agents (ketoconazole, verapamil, diazepam, quinidine, dexamethasone, cyclosporin, teniposide, etoposide, and vincristine), but the concentrations used exceeded those found *in vivo* following normal therapeutic doses. Testosterone, 17α-ethinyl estradiol, retinoic acid, and quercetin, a specific inhibitor of CYP2C8, also inhibited the formation of 6α-hydroxypaclitaxel *in vitro*. The pharmacokinetics of paclitaxel may also be altered *in vivo* as a result of interactions with compounds that are substrates, inducers, or inhibitors of CYP2C8 and/or CYP3A4 *[see Drug Interactions (7)]*.

Excretion

After a 30-minute infusion of 260 mg/m^2 doses of ABRAXANE, the mean values for cumulative urinary recovery of unchanged drug (4%) indicated extensive non-renal clearance. Less than 1% of the total administered dose was excreted in urine as the metabolites 6α-hydroxypaclitaxel and 3'-p-hydroxypaclitaxel.
Fecal excretion was approximately 20% of the total dose administered.

Effect of Hepatic Impairment

The pharmacokinetic profile of ABRAXANE administered as a 30-minute infusion was evaluated in 15 out of 30 solid tumor patients with mild to severe hepatic impairment defined by serum bilirubin levels and AST levels. Patients with AST > 10 x ULN and bilirubin > 5.0 x ULN were not enrolled. ABRAXANE doses were assigned based on the degree of hepatic impairment as described:

- Mild (bilirubin > ULN to ≤ 1.25 x ULN and AST > ULN and < 10 x ULN): 260 mg/m^2
- Moderate (bilirubin 1.26 to 2.0 x ULN and AST > ULN and < 10 x ULN): 200 mg/m^2
- Severe (bilirubin 2.01 to 5.0 x ULN and AST > ULN and < 10 x ULN): 130 mg/m^2

The 260 mg/m^2 dose for mild impairment and the 200 mg/m^2 dose for moderate hepatic impairment adjusted the paclitaxel exposure to the range seen in patients with normal hepatic function (mean AUC0-∞ = 14789 ± 6703). The 130 mg/m^2 dose in patients with severe hepatic impairment resulted in lower paclitaxel exposures than those seen in normal subjects. In addition, patients with severe hepatic impairment had higher mean cycle 1 absolute neutrophil count (ANC) nadir values than those with mild and moderate hepatic impairment.

Table 3: Exposure ($AUC_{0-\infty}$) of ABRAXANE Administered IV over 30 Minutes in Patients with Hepatic Impairment

	Mild (n=5)	Moderate (n=5)	Severe[a] (n=5)
Dose	**260 mg/m^2**	**200 mg/m^2**	**130 mg/m^2**
AUC_{inf} (hr*ng/mL)			
Mean ± SD	17434 ± 11454	14159 ± 13346	9187 ± 6475
Median (range)	13755 (7618, 35262)	7866 (5919, 37613)	6134 (5627, 20684)

[a] bilirubin 2.01 to 5.0 x ULN and AST > ULN and < 10 x ULN

A starting dose of 130 mg/m^2 is recommended in patients with severe hepatic impairment. Escalation of the dose up to 200 mg/m^2 should be considered for subsequent cycles in patients with severe hepatic impairment based on individual tolerance. The 200 mg/m^2 dose has not been evaluated in patients with severe hepatic impairment, but it is predicted to adjust the paclitaxel AUC to the range observed in patients with normal hepatic function. There are no data for patients with AST > 10 x ULN and bilirubin > 5.0 x ULN *[see Dosage and Administration (2.2), and Use in Specific Populations (8.6)]*.

Effect of Renal Impairment

The effect of renal impairment on the disposition of ABRAXANE has not been investigated *[see Use in Specific Populations (8.7)]*.

Drug Interactions
Possible interactions of paclitaxel with concomitantly administered medications have not been formally investigated.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility
The carcinogenic potential of ABRAXANE has not been studied.

Paclitaxel was clastogenic *in vitro* (chromosome aberrations in human lymphocytes) and *in vivo* (micronucleus test in mice). ABRAXANE was not mutagenic in the Ames test or the CHO/HGPRT gene mutation assay.

Administration of paclitaxel protein-bound particles to male rats at 42 mg/m^2 on a weekly basis (approximately 16% of the daily maximum recommended human exposure on a body surface area basis) for 11 weeks prior to mating with untreated female rats resulted in significantly reduced fertility accompanied by decreased pregnancy rates and increased loss of embryos in mated females. A low incidence of skeletal and soft tissue fetal anomalies was also observed at doses of 3 and 12 mg/m^2/week in this study (approximately 1 to 5% of the daily maximum recommended human exposure on a mg/m^2 basis). Testicular atrophy/degeneration was observed in single-dose toxicology studies in rodents administered paclitaxel protein-bound particles at doses lower than the recommended human dose; doses were 54 mg/m^2 in rodents and 175 mg/m^2 in dogs.

14 CLINICAL STUDIES

14.1 Metastatic Breast Carcinoma
Data from 106 patients accrued in two single arm open label studies and from 460 patients enrolled in a randomized comparative study were available to support the use of ABRAXANE in metastatic breast cancer.

Single Arm Open Label Studies
In one study, ABRAXANE was administered as a 30-minute infusion at a dose of 175 mg/m^2 to 43 patients with metastatic breast cancer. The second trial utilized a dose of 300 mg/m^2 as a 30 minute infusion in 63 patients with metastatic breast cancer. Cycles were administered at 3 week intervals. Objective responses were observed in both studies.

Randomized Comparative Study
This multicenter trial was conducted in 460 patients with metastatic breast cancer. Patients were randomized to receive ABRAXANE at a dose of 260 mg/m^2 given as a 30-minute infusion, or paclitaxel injection at 175 mg/m^2 given as a 3-hour infusion. Sixty-four percent of patients had impaired performance status (ECOG 1 or 2) at study entry; 79% had visceral metastases; and 76% had > 3 sites of metastases. Fourteen percent of the patients had not received prior chemotherapy; 27% had received chemotherapy in the adjuvant setting, 40% in the metastatic setting and 19% in both metastatic and adjuvant settings. Fifty-nine percent received study drug as second or greater than second-line therapy. Seventy-seven percent of the patients had been previously exposed to anthracyclines.

In this trial, patients in the ABRAXANE treatment arm had a statistically significantly higher reconciled target lesion response rate (the trial primary endpoint) of 21.5% (95% CI: 16.2% to 26.7%), compared to 11.1% (95% CI: 6.9% to 15.1%) for patients in the paclitaxel injection treatment arm. See Table 4. There was no statistically significant difference in overall survival between the two study arms.

Table 4: Efficacy Results from Randomized Trial

		ABRAXANE 260 mg/m^2	**Paclitaxel Injection 175 mg/m^2**
Reconciled Target Lesion Response Rate (primary endpoint) [a]			
All randomized patients	Response Rate [95% CI]	50/233 (21.5%) [16.19% – 26.73%]	25/227 (11.1%) [6.94% – 15.09%]
	p-value [b]	0.003	
Patients who had failed combination chemotherapy or relapsed within 6 months of adjuvant chemotherapy[c]	Response Rate [95% CI]	20/129 (15.5%) [9.26% – 21.75%]	12/143 (8.4%) [3.85% – 12.94%]

[a] Reconciled Target Lesion Response Rate (TLRR) was the prospectively defined protocol specific endpoint, based on independent radiologic assessment of tumor responses reconciled with investigator responses (which also included clinical information) for the first 6 cycles of therapy. The reconciled TLRR was lower than the investigator Reported Response Rates, which are based on all cycles of therapy.
[b] From Cochran-Mantel-Haenszel test stratified by 1st line vs. > 1st line therapy.
[c] Prior therapy included an anthracycline unless clinically contraindicated.

15 REFERENCES

1. NIOSH Alert: Preventing occupational exposures to antineoplastic and other hazardous drugs in healthcare settings. 2004. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2004-165.

2. OSHA Technical Manual, TED 1-0.15A, Section VI: Chapter 2. Controlling Occupational Exposure to Hazardous Drugs. OSHA, 1999. http://www.osha.gov/dts/osta/otm/otm_vi/otm_vi_2.html

3. American Society of Health-System Pharmacists. (2006) ASHP Guidelines on Handling Hazardous Drugs. *Am J Health-Syst Pharm.* 2006;63:1172-1193.

4. Polovich, M., White, J. M., & Kelleher, L.O. (eds.) 2005. Chemotherapy and biotherapy guidelines and recommendations for practice (2nd. ed.) Pittsburgh, PA: Oncology Nursing Society.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

Product No.: 103450
NDC No.: 68817-134-50 100 mg of paclitaxel in a single use vial, individually packaged in a carton.

16.2 Storage

Store the vials in original cartons at 20°C to 25°C (68° F to 77°F). Retain in the original package to protect from bright light.

16.3 Handling and Disposal

Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published *[see References (15)]*. There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate.

17 PATIENT COUNSELING INFORMATION

See FDA-Approved Patient Labeling.

- Abraxane injection may cause fetal harm. Advise patients to avoid becoming pregnant while receiving this drug. Women of childbearing potential should use effective contraceptives *[see Warnings and Precautions (5.5) and Use in Specific Populations (8.1)]*.
- Men should be advised not to father a child while receiving Abraxane *[see Warnings and Precautions (5.6)]*.
- Patients must be informed of the risk of low blood cell counts and instructed to contact their physician immediately for fever or evidence of infection.
- Patients should be instructed to contact their physician for persistent vomiting, diarrhea, signs of dehydration, cough or breathing difficulties, or signs of an allergic reaction.
- Patients must be informed that sensory neuropathy occurs frequently with Abraxane and patients should advise their physicians of numbness, tingling, pain or weakness involving the extremities *[see Warnings and Precautions (5.2)]*.
- Explain to patients that alopecia,fatigue/asthenia, and myalgia/arthralgia occur frequently with ABRAXANE.

Manufactured for: Celgene Corporation
Summit, NJ 07901

U.S. Patent Numbers: 5,439,686; 5,498,421; 6,096,331; 6,506,405; 6,537,579; 6,749,868; 6,753,006; 7,820,788; 7,923,536; and RE41,884

Patient Information

ABRAXANE® for Injectable Suspension (ah-BRAKS-ane)
(paclitaxel protein-bound particles for injectable suspension) (albumin-bound)

Read this Patient Information before you start receiving ABRAXANE and before each infusion. This information does not take the place of talking with your doctor about your medical condition or your treatment.

What is ABRAXANE?

ABRAXANE is a prescription cancer medicine used to treat advanced breast cancer.

It is not known if ABRAXANE is safe or effective in children.

Who should not receive ABRAXANE?

Do not receive ABRAXANE if:

- your white blood cell count is below 1,500 cells/ mm^3.
- you have had a severe hypersensitivity reaction to ABRAXANE

What should I tell my doctor before receiving ABRAXANE?
Before you receive ABRAXANE, tell your doctor if you:

- have liver or kidney problems
- are a man planning to father a child. You should not father a child during your treatment with ABRAXANE. ABRAXANE can harm the unborn baby of your partner. Talk to your doctor if this is a concern to you.
- are pregnant or plan to become pregnant. ABRAXANE can harm your unborn baby. Women who may become pregnant should use effective birth control (contraception). Talk to your doctor about the best way to prevent pregnancy while receiving ABRAXANE.
- are breastfeeding or plan to breastfeed. It is not known if ABRAXANE passes into your breast milk. You and your doctor should decide if you will receive ABRAXANE or breastfeed.

Tell your doctor about all the medicines you take, including prescription and non-prescription medicines, vitamins, and herbal supplements.

Know the medicines you take. Keep a list to show your doctor and pharmacist each time you get a new medicine.

How will I receive ABRAXANE?
- Your doctor will prescribe ABRAXANE in an amount that is right for you.
- Premedication to prevent allergic reactions is not needed to receive ABRAXANE.
- ABRAXANE will be given to you by intravenous (IV) infusion into your vein.
- Your doctor should do regular blood tests while you receive ABRAXANE.

What are the possible side effects of ABRAXANE?

ABRAXANE may cause serious side effects, including:
- decreased blood cell counts. ABRAXANE can cause a severe decrease in neutrophils (a type of white blood cells important in fighting in bacterial infections) and platelets (important for clotting and to control bleeding). Your doctor will check your blood cell count during your treatment with ABRAXANE and after you have stopped your treatment.
- numbness, tingling, or burning in your hands or feet (neuropathy).

The most common side effects of ABRAXANE include:

- hair loss
- numbness or tingling in the hands or feet
- abnormal heart beat
- tiredness
- joint and muscle pain
- changes in your liver function tests
- low red blood cell count (anemia). Tell your doctor if you feel weak, tired or short of breath.
- nausea
- infections. If you have a fever (temperature of greater than 100.4° F) or other signs of infection, tell your doctor right away.
- diarrhea

Call your doctor for medical advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

General information about the safe and effective use of ABRAXANE.

Medicines are sometimes prescribed for purposes other than those listed in a Patient Information leaflet.

This Patient Information leaflet summarizes the important information about ABRAXANE. If you would like more information, talk to your doctor. You can ask your doctor or pharmacist for information about ABRAXANE that is written for healthcare professionals.

For more information, call 1-800-423-5436.

What are the ingredients in ABRAXANE?

Active ingredient: paclitaxel (bound to human albumin).

Other ingredient: human albumin (containing sodium caprylate and sodium acetyltryptophanate)

This Patient Information has been approved by the U.S. Food and Drug Administration.

Revised: December 2011

Manufactured for: Celgene Corporation
Summit, NJ 07901

U.S. Patent Numbers: 5,439,686; 5,498,421; 6,096,331; 6,506,405; 6,537,579; 6,749,868; 6,753,006 7,820,788; 7,923,536; and RE41,884

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use ABRAXANE safely and effectively. See full prescribing information for ABRAXANE.

ABRAXANE® for Injectable Suspension (paclitaxel protein-bound particles for injectable suspension) (albumin-bound)
Initial U.S. Approval: 2005

WARNING: NEUTROPENIA
See full prescribing information for complete boxed warning.

- **Do not administer ABRAXANE therapy to patients with baseline neutrophil counts of less than 1,500 cells/mm^3. (4)**
- **It is recommended that frequent peripheral blood cell counts be performed to monitor the occurrence of bone marrow suppression. (4, 5.1, 6.1, 6.2, 6.3)**

DO NOT SUBSTITUTE FOR OR WITH OTHER PACLITAXEL FORMULATIONS.

---------- RECENT MAJOR CHANGES ----------

• Dosage and Administration (2.4, 2.8)	12/2014
• Dosage and Administration (2.7)	07/2015
• Warnings and Precautions, Hepatic Impairment (5.6)	12/2014

---------- INDICATIONS AND USAGE ----------

ABRAXANE is a microtubule inhibitor indicated for the treatment of:

- Metastatic breast cancer, after failure of combination chemotherapy for metastatic disease or relapse within 6 months of adjuvant chemotherapy. Prior therapy should have included an anthracycline unless clinically contraindicated. (1.1)
- Locally advanced or metastatic non-small cell lung cancer (NSCLC), as first-line treatment in combination with carboplatin, in patients who are not candidates for curative surgery or radiation therapy. (1.2)
- Metastatic adenocarcinoma of the pancreas as first-line treatment, in combination with gemcitabine. (1.3)

---------- DOSAGE AND ADMINISTRATION ----------

- Metastatic Breast Cancer: Recommended dosage of ABRAXANE is 260 mg/m^2 intravenously over 30 minutes every 3 weeks. (2.1)
- Non-Small Cell Lung Cancer: Recommended dosage of ABRAXANE is 100 mg/m^2 intravenously over 30 minutes on Days 1, 8, and 15 of each 21-day cycle; administer carboplatin on Day 1 of each 21-day cycle immediately after ABRAXANE. (2.2)
- Adenocarcinoma of the Pancreas: Recommended dosage of ABRAXANE is 125 mg/m^2 intravenously over 30-40 minutes on Days 1, 8 and 15 of each 28-day cycle; administer gemcitabine on Days 1, 8 and 15 of each 28-day cycle immediately after ABRAXANE. (2.3)
- Do not administer ABRAXANE to any patient with AST > 10 x ULN or bilirubin > 5 x ULN. Do not administer ABRAXANE to patients with metastatic adenocarcinoma of the pancreas who have moderate to severe hepatic impairment. For diseases other than metastatic adenocarcinoma of the pancreas, reduce starting dose in patients with moderate to severe hepatic impairment. (2.4)
- Dose Reductions: Dose reductions or discontinuation may be needed based on severe hematologic, neurologic, cutaneous, or gastrointestinal toxicities. (2.5)
- Use caution when handling cytotoxic drugs. Closely monitor the infusion site for extravasation and infiltration. No premedication is required prior to administration. (2.6)

---------- DOSAGE FORMS AND STRENGTHS ----------

- For injectable suspension: lyophilized powder containing 100 mg of paclitaxel formulated as albumin-bound particles in single-use vial for reconstitution. (3)

---------- CONTRAINDICATIONS ----------

- Neutrophil counts of < 1,500 cells/mm^3. (4)
- Severe hypersensitivity reaction to ABRAXANE. (4)

---------- WARNINGS AND PRECAUTIONS ----------

- ABRAXANE causes myelosuppression. Monitor CBC and withhold and/or reduce the dose as needed. (5.1)
- Sensory neuropathy occurs frequently and may require dose reduction or treatment interruption. (5.2)
- Sepsis occurred in patients with or without neutropenia who received ABRAXANE in combination with gemcitabine; interrupt ABRAXANE and gemcitabine until sepsis resolves, and if neutropenia, until neutrophils are at least 1500 cells/mm^3, then resume treatment at reduced dose levels. (5.3)
- Pneumonitis occurred with the use of ABRAXANE in combination with gemcitabine; permanently discontinue treatment with ABRAXANE and gemcitabine. (5.4)
- Severe hypersensitivity reactions with fatal outcome have been reported. Do not re-challenge with this drug. (5.5)
- Exposure and toxicity of paclitaxel can be increased in patients with hepatic impairment; therefore administer with caution. (5.6)
- ABRAXANE contains albumin derived from human blood, which has a theoretical risk of viral transmission. (5.7)
- Fetal harm may occur when administered to a pregnant woman. Advise women of childbearing potential to avoid becoming pregnant while receiving ABRAXANE. (5.8)
- Advise men not to father a child while on ABRAXANE. (5.9)

---------- ADVERSE REACTIONS ----------

- The most common adverse reactions (≥ 20%) in metastatic breast cancer are alopecia, neutropenia, sensory neuropathy, abnormal ECG, fatigue/asthenia, myalgia/arthralgia, AST elevation, alkaline phosphatase elevation, anemia, nausea, infections, and diarrhea. (6.1)
- The most common adverse reactions (≥ 20%) in NSCLC are anemia, neutropenia, thrombocytopenia, alopecia, peripheral neuropathy, nausea, and fatigue. (6.2)
- The most common (≥ 20%) adverse reactions of ABRAXANE in adenocarcinoma of the pancreas are neutropenia, fatigue, peripheral neuropathy, nausea, alopecia, peripheral edema, diarrhea, pyrexia, vomiting, decreased appetite, rash, and dehydration. (6.3)

To report SUSPECTED ADVERSE REACTIONS, contact Celgene Corporation at 1-888-423-5436 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

---------- DRUG INTERACTIONS ----------

- Use caution when concomitantly administering ABRAXANE with inhibitors or inducers of either CYP2C8 or CYP3A4. (7)

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling.

Revised: 07/2015

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FULL PRESCRIBING INFORMATION
ABRAXANE® for Injectable Suspension (paclitaxel protein-bound particles for injectable suspension) (albumin-bound)

WARNING: NEUTROPENIA

- **Do not administer ABRAXANE therapy to patients who have baseline neutrophil counts of less than 1,500 cells/mm^3. In order to monitor the occurrence of bone marrow suppression, primarily neutropenia, which may be severe and result in infection, it is recommended that frequent peripheral blood cell counts be performed on all patients receiving ABRAXANE *[see Contraindications (4), Warnings and Precautions (5.1) and Adverse Reactions (6.1, 6.2, 6.3)].***
- **Note: An albumin form of paclitaxel may substantially affect a drug's functional properties relative to those of drug in solution. DO NOT SUBSTITUTE FOR OR WITH OTHER PACLITAXEL FORMULATIONS.**

1 INDICATIONS AND USAGE

1.1 Metastatic Breast Cancer
ABRAXANE is indicated for the treatment of breast cancer after failure of combination chemotherapy for metastatic disease or relapse within 6 months of adjuvant chemotherapy. Prior therapy should have included an anthracycline unless clinically contraindicated.

1.2 Non-Small Cell Lung Cancer
ABRAXANE is indicated for the first-line treatment of locally advanced or metastatic non-small cell lung cancer, in combination with carboplatin, in patients who are not candidates for curative surgery or radiation therapy.

1.3 Adenocarcinoma of the Pancreas
ABRAXANE is indicated for the first-line treatment of patients with metastatic adenocarcinoma of the pancreas, in combination with gemcitabine.

2 DOSAGE AND ADMINISTRATION

2.1 Metastatic Breast Cancer
After failure of combination chemotherapy for metastatic breast cancer or relapse within 6 months of adjuvant chemotherapy, the recommended regimen for ABRAXANE is 260 mg/m^2 administered intravenously over 30 minutes every 3 weeks.

2.2 Non-Small Cell Lung Cancer
The recommended dose of ABRAXANE is 100 mg/m^2 administered as an intravenous infusion over 30 minutes on Days 1, 8, and 15 of each 21-day cycle. Administer carboplatin on Day 1 of each 21 day cycle immediately after ABRAXANE *[see Clinical Studies (14.2)]*.

2.3 Adenocarcinoma of the Pancreas
The recommended dose of ABRAXANE is 125 mg/m^2 administered as an intravenous infusion over 30-40 minutes on Days 1, 8 and 15 of each 28-day cycle. Administer gemcitabine immediately after ABRAXANE on Days 1, 8 and 15 of each 28-day cycle *[see Clinical Studies (14.3)]*.

2.4 Dosage in Patients with Hepatic Impairment
For patients with mild hepatic impairment (total bilirubin greater than ULN and less than or equal to 1.5 x ULN and aspartate aminotransferase [AST] less than or equal to 10 x ULN), no dose adjustments are required, regardless of indication.

Do not administer ABRAXANE to patients with metastatic adenocarcinoma of the pancreas who have moderate to severe hepatic impairment.

Do not administer ABRAXANE to patients with total bilirubin greater than 5 x ULN or AST greater than 10 x ULN regardless of indication as these patients have not been studied.

Recommendations for dosage adjustment for the first course of therapy are shown in Table 1.

Table 1: Recommendations for Starting Dose in Patients with Hepatic Impairment

	SGOT (AST) Levels		Bilirubin Levels	ABRAXANE Dose[a]		
				MBC	NSCLC[c]	Pancreatic[c] Adenocarcinoma
Mild	< 10 x ULN	AND	> ULN to ≤ 1. 5 x ULN	260 mg/m^2	100 mg/m^2	125 mg/m^2
Moderate	< 10 x ULN	AND	> 1.5 to ≤ 3 x ULN	200 mg/m^2 [b]	80 mg/m^2 [b]	not recommended
Severe	< 10 x ULN	AND	> 3 to ≤ 5 x ULN	200 mg/m^2 [b]	80 mg/m^2 [b]	not recommended
	> 10 x ULN	OR	> 5 x ULN	not recommended	not recommended	not recommended

MBC = Metastatic Breast Cancer; NSCLC = Non-Small Cell Lung Cancer.

[a] Dosage recommendations are for the first course of therapy. The need for further dose adjustments in subsequent courses should be based on individual tolerance.

[b] A dose increase to 260 mg/m^2 for patients with metastatic breast cancer or 100 mg/m^2 for patients with non-small cell lung cancer in subsequent courses should be considered if the patient tolerates the reduced dose for two cycles.

[c] Patients with bilirubin levels above the upper limit of normal were excluded from clinical trials for pancreatic or lung cancer.

2.5 Dose Reduction/Discontinuation Recommendations

Metastatic Breast Cancer

Patients who experience severe neutropenia (neutrophils less than 500 cells/mm^3 for a week or longer) or severe sensory neuropathy during ABRAXANE therapy should have dosage reduced to 220 mg/m^2 for subsequent courses of ABRAXANE. For recurrence of severe neutropenia or severe sensory neuropathy, additional dose reduction should be made to 180 mg/m^2. For Grade 3 sensory neuropathy hold treatment until resolution to Grade 1 or 2, followed by a dose reduction for all subsequent courses of ABRAXANE *[see Contraindications (4), Warnings and Precautions (5.1, 5.2) and Adverse Reactions (6.1)]*.

Non-Small Cell Lung Cancer

- Do not administer ABRAXANE on Day 1 of a cycle until absolute neutrophil count (ANC) is at least 1500 cells/mm^3 and platelet count is at least 100,000 cells/mm^3 *[see Contraindications (4), Warnings and Precautions (5.1) and Adverse Reactions (6.2)]*.
- In patients who develop severe neutropenia or thrombocytopenia withhold treatment until counts recover to an absolute neutrophil count of at least 1500 cells/mm^3 and platelet count of at least 100,000 cells/mm^3 on Day 1 or to an absolute neutrophil count of at least 500 cells/mm^3 and platelet count of at least 50,000 cells/mm^3 on Days 8 or 15 of the cycle. Upon resumption of dosing, permanently reduce ABRAXANE and carboplatin doses as outlined in Table 2.
- Withhold ABRAXANE for Grade 3-4 peripheral neuropathy. Resume ABRAXANE and carboplatin at reduced doses (see Table 2) when peripheral neuropathy improves to Grade 1 or completely resolves *[see Warnings and Precautions (5.2) and Adverse Reactions (6.2)]*.

Table 2: Permanent Dose Reductions for Hematologic and Neurologic Adverse Drug Reactions in NSCLC

Adverse Drug Reaction	Occurrence	Weekly ABRAXANE Dose (mg/m^2)	Every 3-Week Carboplatin Dose (AUC mg•min/mL)
Neutropenic Fever (ANC less than 500/mm^3 with fever >38°C) OR Delay of next cycle by more than 7 days for ANC less than 1500/mm^3 OR ANC less than 500/mm^3 for more than 7 days	First	75	4.5
	Second	50	3
	Third	Discontinue Treatment	
Platelet count less than 50,000/mm^3	First	75	4.5
	Second	Discontinue Treatment	
Severe sensory Neuropathy – Grade 3 or 4	First	75	4.5
	Second	50	3
	Third	Discontinue Treatment	

Adenocarcinoma of the Pancreas
Dose level reductions for patients with adenocarcinoma of the pancreas, as referenced in Tables 4 and 5, are provided in Table 3.

Table 3: Dose Level Reductions for Patients with Adenocarcinoma of the Pancreas

Dose Level	ABRAXANE (mg/m^2)	Gemcitabine (mg/m^2)
Full dose	125	1000
1st dose reduction	100	800
2nd dose reduction	75	600
If additional dose reduction required	Discontinue	Discontinue

Recommended dose modifications for neutropenia and thrombocytopenia for patients with adenocarcinoma of the pancreas are provided in Table 4.

Table 4: Dose Recommendation and Modifications for Neutropenia and/or Thrombocytopenia at the Start of a Cycle or within a Cycle for Patients with Adenocarcinoma of the Pancreas

Cycle Day	ANC ($cells/mm^3$)		Platelet count ($cells/mm^3$)	ABRAXANE / Gemcitabine
Day 1	< 1500	OR	< 100,000	Delay doses until recovery
Day 8	500 to < 1000	OR	50,000 to < 75,000	Reduce 1 dose level
	< 500	OR	< 50,000	Withhold doses
Day 15: If Day 8 doses were reduced or given without modification:				
	500 to < 1000	OR	50,000 to < 75,000	Reduce 1 dose level from Day 8
	< 500	OR	< 50,000	Withhold doses
Day 15: If Day 8 doses were withheld:				
	≥ 1000	OR	≥ 75,000	Reduce 1 dose level from Day 1
	500 to < 1000	OR	50,000 to < 75,000	Reduce 2 dose levels from Day 1
	< 500	OR	< 50,000	Withhold doses

ANC = Absolute Neutrophil Count

Recommended dose modifications for other adverse drug reactions in patients with adenocarcinoma of the pancreas are provided in Table 5.

Table 5: Dose Modifications for Other Adverse Drug Reactions in Patients with Adenocarcinoma of the Pancreas

Adverse Drug Reaction	ABRAXANE	Gemcitabine
Febrile Neutropenia: Grade 3 or 4	Withhold until fever resolves and ANC ≥ 1500; resume at next lower dose level	
Peripheral Neuropathy: Grade 3 or 4	Withhold until improves to ≤ Grade 1; resume at next lower dose level	No dose reduction
Cutaneous Toxicity: Grade 2 or 3	Reduce to next lower dose level; discontinue treatment if toxicity persists	
Gastrointestinal Toxicity: Grade 3 mucositis or diarrhea	Withhold until improves to ≤ Grade 1; resume at next lower dose level	

2.6 Preparation and Administration Precautions
ABRAXANE is a cytotoxic drug and, as with other potentially toxic paclitaxel compounds, caution should be exercised in handling ABRAXANE. The use of gloves is recommended. If ABRAXANE (lyophilized cake or reconstituted suspension) contacts the skin, wash the skin immediately and thoroughly with soap and water. Following topical exposure to paclitaxel, events may include tingling, burning and redness. If ABRAXANE contacts mucous membranes, the membranes should be flushed thoroughly with water.

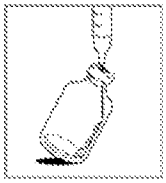
Given the possibility of extravasation, it is advisable to closely monitor the infusion site for possible infiltration during drug administration. Limiting the infusion of ABRAXANE to 30 minutes, as directed, reduces the likelihood of infusion-related reactions *[see Adverse Reactions (6.4)]*.

Premedication to prevent hypersensitivity reactions is generally not needed prior to the administration of ABRAXANE. Premedication may be needed in patients who have had prior hypersensitivity reactions to ABRAXANE. Patients who experience a severe hypersensitivity reaction to ABRAXANE should not be re-challenged with this drug [*see Warnings and Precautions (5.5)*].

2.7 Preparation for Intravenous Administration

ABRAXANE is supplied as a sterile lyophilized powder for reconstitution before use. **AVOID ERRORS, READ ENTIRE PREPARATION INSTRUCTIONS PRIOR TO RECONSTITUTION**.

1. Aseptically, reconstitute each vial by injecting 20 mL of 0.9% Sodium Chloride Injection, USP.
2. Slowly inject the 20 mL of 0.9% Sodium Chloride Injection, USP, over a minimum of 1 minute, using the sterile syringe to direct the solution flow onto the INSIDE WALL OF THE VIAL.



3. DO NOT INJECT the 0.9% Sodium Chloride Injection, USP, directly onto the lyophilized cake as this will result in foaming.
4. Once the injection is complete, allow the vial to sit for a minimum of 5 minutes to ensure proper wetting of the lyophilized cake/powder.
5. Gently swirl and/or invert the vial slowly for at least 2 minutes until complete dissolution of any cake/powder occurs. Avoid generation of foam.
6. If foaming or clumping occurs, stand solution for at least 15 minutes until foam subsides.

Each mL of the reconstituted formulation will contain 5 mg/mL paclitaxel.

The reconstituted suspension should be milky and homogenous without visible particulates. If particulates or settling are visible, the vial should be **gently** inverted again to ensure complete resuspension prior to use. Discard the reconstituted suspension if precipitates are observed. Discard any unused portion.

Calculate the exact total dosing volume of 5 mg/mL suspension required for the patient and slowly withdraw the dosing volume of the reconstituted suspension from the vial(s) into a syringe: Dosing volume (mL)=Total dose (mg)/5 (mg/mL).

Inject the appropriate amount of reconstituted ABRAXANE into an empty, sterile intravenous bag [plasticized polyvinyl chloride (PVC) containers, PVC or non-PVC type intravenous bag]. The use of specialized DEHP-free solution containers or administration sets is not necessary to prepare or administer ABRAXANE infusions. The use of medical devices containing silicone oil as a lubricant (ie, syringes and intravenous bags) to reconstitute and administer ABRAXANE may result in the formation of proteinaceous strands.

Visually inspect the reconstituted ABRAXANE suspension in the intravenous bag prior to administration. Discard the reconstituted suspension if proteinaceous strands, particulate matter or discoloration are observed.

2.8 Stability

Unopened vials of ABRAXANE are stable until the date indicated on the package when stored between 20ºC to 25ºC (68ºF to 77ºF) in the original package. Neither freezing nor refrigeration adversely affects the stability of the product.

Stability of Reconstituted Suspension in the Vial

Reconstituted ABRAXANE in the vial should be used immediately, but may be refrigerated at 2ºC to 8ºC (36ºF to 46ºF) for a maximum of 24 hours if necessary. If not used immediately, each vial of reconstituted suspension should be replaced in the original carton to protect it from bright light. Discard any unused portion.

Stability of Reconstituted Suspension in the Infusion Bag

The suspension for infusion when prepared as recommended in an infusion bag should be used immediately, but may be refrigerated at 2°C to 8°C (36°F to 46°F) and protected from bright light for a maximum of 24 hours.

The total combined refrigerated storage time of reconstituted ABRAXANE in the vial and in the infusion bag is 24 hours. This may be followed by storage in the infusion bag at ambient temperature (approximately 25°C) and lighting conditions for a maximum of 4 hours.

Discard any unused portion.

3 DOSAGE FORMS AND STRENGTHS

For injectable suspension: lyophilized powder containing 100 mg of paclitaxel formulated as albumin-bound particles in single-use vial for reconstitution.

4 CONTRAINDICATIONS

- ABRAXANE should not be used in patients who have baseline neutrophil counts of < 1,500 cells/mm^3.
- Patients who experience a severe hypersensitivity reaction to ABRAXANE should not be rechallenged with the drug.

5 WARNINGS AND PRECAUTIONS

5.1 Hematologic Effects

Bone marrow suppression (primarily neutropenia) is dose-dependent and a dose-limiting toxicity of ABRAXANE. In clinical studies, Grade 3-4 neutropenia occurred in 34% of patients with metastatic breast cancer (MBC), 47% of patients with non-small cell lung cancer (NSCLC), and 38% of patients with pancreatic cancer.

Monitor for myelotoxicity by performing complete blood cell counts frequently, including prior to dosing on Day 1 (for MBC) and Days 1, 8, and 15 (for NSCLC and for pancreatic cancer). Do not administer ABRAXANE to patients with baseline absolute neutrophil counts (ANC) of less than 1,500 cells/mm^3. In the case of severe neutropenia (<500 cells/mm^3 for seven days or more) during a course of ABRAXANE therapy, reduce the dose of ABRAXANE in subsequent courses in patients with either MBC or NSCLC.

In patients with MBC, resume treatment with every-3-week cycles of ABRAXANE after ANC recovers to a level >1,500 cells/mm^3 and platelets recover to a level >100,000 cells/mm^3.

In patients with NSCLC, resume treatment if recommended (see Dosage and Administration, Table 2) at permanently reduced doses for both weekly ABRAXANE and every-3-week carboplatin after ANC recovers to at least 1500 cells/mm^3 and platelet count of at least 100,000 cells/mm^3 on Day 1 or to an ANC of at least 500 cells/mm^3 and platelet count of at least 50,000 cells/mm^3 on Days 8 or 15 of the cycle *[see Dosage and Administration (2.5)]*.

In patients with adenocarcinoma of the pancreas, withhold ABRAXANE and gemcitabine if the ANC is less than 500 cells/mm^3 or platelets are less than 50,000 cells/mm^3 and delay initiation of the next cycle if the ANC is less than 1500 cells/mm^3 or platelet count is less than 100,000 cells/mm^3 on Day 1 of the cycle. Resume treatment with appropriate dose reduction if recommended *[see Dosage and Administration (2.5)]*.

5.2 Nervous System

Sensory neuropathy is dose- and schedule-dependent *[see Adverse Reactions (6.1, 6.2, 6.3)]*. The occurrence of Grade 1 or 2 sensory neuropathy does not generally require dose modification. If ≥ Grade 3 sensory neuropathy develops, withhold ABRAXANE treatment until resolution to Grade 1 or 2 for metastatic breast cancer or until resolution to ≤ Grade 1 for NSCLC and pancreatic cancer followed by a dose reduction for all subsequent courses of ABRAXANE *[see Dosage and Administration (2.5)]*.

5.3 Sepsis

Sepsis occurred in 5% of patients with or without neutropenia who received ABRAXANE in combination with gemcitabine. Biliary obstruction or presence of biliary stent were risk factors for severe or fatal sepsis. If a patient becomes febrile (regardless of ANC) initiate treatment with broad spectrum antibiotics. For febrile neutropenia, interrupt ABRAXANE and gemcitabine until fever resolves and ANC ≥ 1500, then resume treatment at reduced dose levels *[see Dosage and Administration (2.5)]*.

5.4 Pneumonitis

Pneumonitis, including some cases that were fatal, occurred in 4% of patients receiving ABRAXANE in combination with gemcitabine. Monitor patients for signs and symptoms of pneumonitis and interrupt ABRAXANE and gemcitabine during evaluation of suspected pneumonitis. After ruling out infectious etiology and upon making a diagnosis of pneumonitis, permanently discontinue treatment with ABRAXANE and gemcitabine.

5.5 Hypersensitivity

Severe and sometimes fatal hypersensitivity reactions, including anaphylactic reactions, have been reported. Patients who experience a severe hypersensitivity reaction to ABRAXANE should not be rechallenged with this drug.

5.6 Hepatic Impairment
Because the exposure and toxicity of paclitaxel can be increased with hepatic impairment, administration of ABRAXANE in patients with hepatic impairment should be performed with caution. Patients with hepatic impairment may be at increased risk of toxicity, particularly from myelosuppression; such patients should be closely monitored for development of profound myelosuppression. ABRAXANE is not recommended in patients who have total bilirubin >5 x ULN or AST >10 x ULN. In addition, ABRAXANE is not recommended in patients with metastatic adenocarcinoma of the pancreas who have moderate to severe hepatic impairment (total bilirubin >1.5 x ULN and AST ≤10 x ULN). The starting dose should be reduced for patients with moderate or severe hepatic impairment *[see Dosage and Administration (2.4), Use in Specific Populations (8.6) and Clinical Pharmacology (12.3)]*.

5.7 Albumin (Human)
ABRAXANE contains albumin (human), a derivative of human blood. Based on effective donor screening and product manufacturing processes, it carries a remote risk for transmission of viral diseases. A theoretical risk for transmission of Creutzfeldt-Jakob Disease (CJD) also is considered extremely remote. No cases of transmission of viral diseases or CJD have ever been identified for albumin.

5.8 Use in Pregnancy
ABRAXANE can cause fetal harm when administered to a pregnant woman. Administration of paclitaxel formulated as albumin-bound particles to rats during pregnancy at doses lower than the maximum recommended human dose, based on body surface area, caused embryo-fetal toxicities, including intrauterine mortality, increased resorptions, reduced numbers of live fetuses, and malformations.

There are no adequate and well-controlled studies in pregnant women receiving ABRAXANE. If this drug is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant while receiving ABRAXANE *[see Use in Specific Populations (8.1)]*.

5.9 Use in Men
Men should be advised not to father a child while receiving ABRAXANE *[see Nonclinical Toxicology (13.1)]*.

6 ADVERSE REACTIONS
Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The most common adverse reactions (≥ 20%) with single-agent use of ABRAXANE in metastatic breast cancer are alopecia, neutropenia, sensory neuropathy, abnormal ECG, fatigue/asthenia, myalgia/arthralgia, AST elevation, alkaline phosphatase elevation, anemia, nausea, infections, and diarrhea *[see Adverse Reactions (6.1)]*.

The most common adverse reactions (≥ 20%) of ABRAXANE in combination with carboplatin for non-small cell lung cancer are anemia, neutropenia, thrombocytopenia, alopecia, peripheral neuropathy, nausea, and fatigue *[see Adverse Reactions (6.2)]*. The most common serious adverse reactions of ABRAXANE in combination with carboplatin for non-small cell lung cancer are anemia (4%) and pneumonia (3%). The most common adverse reactions resulting in permanent discontinuation of ABRAXANE are neutropenia (3%), thrombocytopenia (3%), and peripheral neuropathy (1%). The most common adverse reactions resulting in dose reduction of ABRAXANE are neutropenia (24%), thrombocytopenia (13%), and anemia (6%). The most common adverse reactions leading to withholding or delay in ABRAXANE dosing are neutropenia (41%), thrombocytopenia (30%), and anemia (16%).

In a randomized open-label trial of ABRAXANE in combination with gemcitabine for pancreatic adenocarcinoma [*see Clinical Studies (14.3)*], the most common (≥ 20%) selected (with a ≥ 5% higher incidence) adverse reactions of ABRAXANE are neutropenia, fatigue, peripheral neuropathy, nausea, alopecia, peripheral edema, diarrhea, pyrexia, vomiting, decreased appetite, rash, and dehydration. The most common serious adverse reactions of ABRAXANE (with a ≥ 1% higher incidence) are pyrexia (6%), dehydration (5%), pneumonia (4%) and vomiting (4%). The most common adverse reactions resulting in permanent discontinuation of ABRAXANE are peripheral neuropathy (8%), fatigue (4%) and thrombocytopenia (2%). The most common adverse reactions resulting in dose reduction of ABRAXANE are neutropenia (10%) and peripheral neuropathy (6%). The most common adverse reactions leading to withholding or delay in ABRAXANE dosing are neutropenia (16%), thrombocytopenia (12%), fatigue (8%), peripheral neuropathy (15%), anemia (5%) and diarrhea (5%).

6.1 Clinical Trials Experience in Metastatic Breast Cancer
Table 6 shows the frequency of important adverse events in the randomized comparative trial for the patients who received either single-agent ABRAXANE or paclitaxel injection for the treatment of metastatic breast cancer.

Table 6: Frequency[a] of Important Treatment Emergent Adverse Events in the Randomized Metastatic Breast Cancer Study on an Every-3-Weeks Schedule

	Percent of Patients	
	ABRAXANE 260 mg/m^2 over 30 min (n=229)	**Paclitaxel Injection 175 mg/m^2 over 3 h[b] (n=225)**
Bone Marrow		
Neutropenia		
< 2.0 x 10^9/L	80	82
< 0.5 x 10^9/L	9	22
Thrombocytopenia		
< 100 x 10^9/L	2	3
< 50 x 10^9/L	<1	<1
Anemia		
< 11 g/dL	33	25
< 8 g/dL	1	<1
Infections	24	20
Febrile Neutropenia	2	1
Neutropenic Sepsis	<1	<1
Bleeding	2	2
Hypersensitivity Reaction[c]		
All	4	12
Severe[d]	0	2
Cardiovascular		
Vital Sign Changes During Administration		
Bradycardia	<1	<1
Hypotension	5	5
Severe Cardiovascular Events[d]	3	4
Abnormal ECG		
All Patients	60	52
Patients with Normal Baseline	35	30
Respiratory		
Cough	7	6
Dyspnea	12	9
Sensory Neuropathy		
Any Symptoms	71	56
Severe Symptoms[d]	10	2
Myalgia / Arthralgia		
Any Symptoms	44	49
Severe Symptoms[d]	8	4
Asthenia		
Any Symptoms	47	39
Severe Symptoms[d]	8	3
Fluid Retention/Edema		
Any Symptoms	10	8
Severe Symptoms[d]	0	<1
Gastrointestinal		
Nausea		
Any Symptoms	30	22
Severe Symptoms[d]	3	<1
Vomiting		
Any Symptoms	18	10
Severe Symptoms[d]	4	1
Diarrhea		
Any Symptoms	27	15
Severe Symptoms[d]	<1	1
Mucositis		
Any Symptoms	7	6
Severe Symptoms[d]	<1	0
Alopecia	90	94
Hepatic (Patients with Normal Baseline)		
Bilirubin Elevations	7	7
Alkaline Phosphatase Elevations	36	31
AST (SGOT) Elevations	39	32

	Percent of Patients	
	ABRAXANE 260 mg/m^2 over 30 min (n=229)	**Paclitaxel Injection 175 mg/m^2 over 3 h[b] (n=225)**
Injection Site Reaction	<1	1

[a] Based on worst grade by NCI Common Terminology Criteria for Adverse Events (CTCAE) version 2.
[b] Paclitaxel injection patients received premedication.
[c] Includes treatment-related events related to hypersensitivity (e.g., flushing, dyspnea, chest pain, hypotension) that began on a day of dosing.
[d] Severe events are defined as at least grade 3 toxicity.

Adverse Event Experiences by Body System

Hematologic Disorders
Neutropenia was dose dependent and reversible. Among patients with metastatic breast cancer in the randomized trial, neutrophil counts declined below 500 cells/mm^3 (Grade 4) in 9% of the patients treated with a dose of 260 mg/m^2 compared to 22% in patients receiving paclitaxel injection at a dose of 175 mg/m^2. Pancytopenia has been observed in clinical trials.

Infections
Infectious episodes were reported in 24% of the patients treated with ABRAXANE. Oral candidiasis, respiratory tract infections and pneumonia were the most frequently reported infectious complications.

Hypersensitivity Reactions (HSRs)
Grade 1 or 2 HSRs occurred on the day of ABRAXANE administration and consisted of dyspnea (1%) and flushing, hypotension, chest pain, and arrhythmia (all <1%). The use of ABRAXANE in patients previously exhibiting hypersensitivity to paclitaxel injection or human albumin has not been studied.

Cardiovascular
Hypotension, during the 30-minute infusion, occurred in 5% of patients. Bradycardia, during the 30-minute infusion, occurred in <1% of patients. These vital sign changes most often caused no symptoms and required neither specific therapy nor treatment discontinuation.

Severe cardiovascular events possibly related to single-agent ABRAXANE occurred in approximately 3% of patients. These events included cardiac ischemia/infarction, chest pain, cardiac arrest, supraventricular tachycardia, edema, thrombosis, pulmonary thromboembolism, pulmonary emboli, and hypertension. Cases of cerebrovascular attacks (strokes) and transient ischemic attacks have been reported.

Electrocardiogram (ECG) abnormalities were common among patients at baseline. ECG abnormalities on study did not usually result in symptoms, were not dose-limiting, and required no intervention. ECG abnormalities were noted in 60% of patients. Among patients with a normal ECG prior to study entry, 35% of all patients developed an abnormal tracing while on study. The most frequently reported ECG modifications were non-specific repolarization abnormalities, sinus bradycardia, and sinus tachycardia.

Respiratory
Dyspnea (12%), cough (7%), and pneumothorax (<1%) were reported after treatment with ABRAXANE.

Neurologic
The frequency and severity of sensory neuropathy increased with cumulative dose. Sensory neuropathy was the cause of ABRAXANE discontinuation in 7/229 (3%) patients. Twenty-four patients (10%) treated with ABRAXANE developed Grade 3 peripheral neuropathy; of these patients, 14 had documented improvement after a median of 22 days; 10 patients resumed treatment at a reduced dose of ABRAXANE and 2 discontinued due to peripheral neuropathy. Of the 10 patients without documented improvement, 4 discontinued the study due to peripheral neuropathy.

No Grade 4 sensory neuropathies were reported. Only one incident of motor neuropathy (Grade 2) was observed in either arm of the controlled trial.

Vision Disorders
Ocular/visual disturbances occurred in 13% of all patients (n=366) treated with ABRAXANE and 1% were severe. The severe cases (keratitis and blurred vision) were reported in patients who received higher doses than those recommended (300 or 375 mg/m^2). These effects generally have been reversible.

Arthralgia/Myalgia
The symptoms were usually transient, occurred two or three days after ABRAXANE administration, and resolved within a few days.

Hepatic
Grade 3 or 4 elevations in GGT were reported for 14% of patients treated with ABRAXANE and 10% of patients treated with paclitaxel injection in the randomized trial.

Renal
Overall 11% of patients experienced creatinine elevation, 1% severe. No discontinuations, dose reductions, or dose delays were caused by renal toxicities.

Other Clinical Events
Nail changes (changes in pigmentation or discoloration of nail bed) have been reported. Edema occurred in 10% of patients; no patients had severe edema. Dehydration and pyrexia were also reported.

6.2 Clinical Trials Experience in Non-Small Cell Lung Cancer
Adverse reactions were assessed in 514 ABRAXANE/carboplatin-treated patients and 524 paclitaxel injection/carboplatin-treated patients receiving first-line systemic treatment for locally advanced (stage IIIB) or metastatic (IV) non-small cell lung cancer (NSCLC) in a multicenter, randomized, open-label trial. ABRAXANE was administered as an intravenous infusion over 30 minutes at a dose of 100 mg/m^2 on Days 1, 8, and 15 of each 21-day cycle. Paclitaxel injection was administered as an intravenous infusion over 3 hours at a dose of 200 mg/m^2, following premedication. In both treatment arms carboplatin at a dose of AUC = 6 mg•min/mL was administered intravenously on Day 1 of each 21-day cycle after completion of ABRAXANE/paclitaxel infusion.
The differences in paclitaxel dose and schedule between the two arms limit direct comparison of dose- and schedule-dependent adverse reactions. Among patients evaluable for adverse reactions, the median age was 60 years, 75% were men, 81% were White, 49% had adenocarcinoma, 43% had squamous cell lung cancer, 76% were ECOG PS 1. Patients in both treatment arms received a median of 6 cycles of treatment.

The following common (≥ 10% incidence) adverse reactions were observed at a similar incidence in ABRAXANE plus carboplatin-treated and paclitaxel injection plus carboplatin-treated patients: alopecia 56%, nausea 27%, fatigue 25%, decreased appetite 17%, asthenia 16%, constipation 16%, diarrhea 15%, vomiting 12%, dyspnea 12%, and rash 10% (incidence rates are for the ABRAXANE plus carboplatin treatment group).

Table 7 provides the frequency and severity of laboratory-detected abnormalities which occurred with a difference of ≥ 5% for all grades (1-4) or ≥ 2% for Grade 3-4 toxicity between ABRAXANE plus carboplatin-treated patients or paclitaxel injection plus carboplatin-treated patients.

Table 7: Selected Hematologic Laboratory-Detected Abnormalities With a Difference of ≥ 5% for grades (1-4) or ≥ 2% for Grade 3-4 Toxicity Between Treatment Groups

	ABRAXANE (100 mg/m^2 weekly) plus carboplatin		Paclitaxel Injection (200 mg/m^2 every 3 weeks) plus carboplatin	
	Grades 1-4 (%)	Grade 3-4 (%)	Grades 1-4 (%)	Grade 3-4 (%)
Anemia[1,2]	98	28	91	7
Neutropenia[1,3]	85	47	83	58
Thrombocytopenia[1,3]	68	18	55	9

[1] 508 patients assessed in ABRAXANE/carboplatin-treated group
[2] 514 patients assessed in paclitaxel injection/carboplatin-treated group
[3] 513 patients assessed in paclitaxel injection/carboplatin-treated group

Table 8 provides the frequency and severity of adverse reactions, which occurred with a difference of ≥ 5% for all grades (1-4) or ≥ 2% for Grade 3-4 between either treatment group for the 514 ABRAXANE plus carboplatin-treated patients compared with the 524 patients who received paclitaxel injection plus carboplatin.

Table 8: Selected Adverse Reactions with a Difference of ≥5% for All Grade Toxicity or ≥2% for Grade 3-4 Toxicity Between Treatment Groups

		ABRAXANE (100 mg/m^2 weekly) + carboplatin (N=514)		Paclitaxel Injection (200 mg/m^2 every 3 weeks) + carboplatin (N=524)	
System Organ Class	MedDRA v 12.1 Preferred Term	Grade 1-4 Toxicity (%)	Grade 3-4 Toxicity (%)	Grades 1-4 Toxicity (%)	Grade 3-4 Toxicity (%)
Nervous system disorders	Peripheral neuropathy[a]	48	3	64	12
General disorders and administration site conditions	Edema peripheral	10	0	4	<1
Respiratory thoracic and mediastinal disorders	Epistaxis	7	0	2	0

System Organ Class	MedDRA v 12.1 Preferred Term	ABRAXANE (100 mg/m^2 weekly) + carboplatin (N=514)		Paclitaxel Injection (200 mg/m^2 every 3 weeks) + carboplatin (N=524)	
		Grade 1-4 Toxicity (%)	Grade 3-4 Toxicity (%)	Grades 1-4 Toxicity (%)	Grade 3-4 Toxicity (%)
Musculoskeletal and connective tissue disorders	Arthralgia	13	<1	25	2
	Myalgia	10	<1	19	2

[a] Peripheral neuropathy is defined by the MedDRA Version 14.0 SMQ neuropathy (broad scope).

For the ABRAXANE plus carboplatin treated group, 17/514 (3%) patients developed Grade 3 peripheral neuropathy and no patients developed Grade 4 peripheral neuropathy. Grade 3 neuropathy improved to Grade 1 or resolved in 10/17 patients (59%) following interruption or discontinuation of ABRAXANE.

6.3 Clinical Trials Experience in Adenocarcinoma of the Pancreas

Adverse reactions were assessed in 421 patients who received ABRAXANE plus gemcitabine and 402 patients who received gemcitabine for the first-line systemic treatment of metastatic adenocarcinoma of the pancreas in a multicenter, multinational, randomized, controlled, open-label trial. Patients received a median treatment duration of 3.9 months in the ABRAXANE/gemcitabine group and 2.8 months in the gemcitabine group. For the treated population, the median relative dose intensity for gemcitabine was 75% in the ABRAXANE/gemcitabine group and 85% in the gemcitabine group. The median relative dose intensity of ABRAXANE was 81%.

Table 9 provides the frequency and severity of laboratory-detected abnormalities which occurred at a higher incidence for Grades 1-4 (≥ 5%) or for Grade 3-4 (≥ 2%) toxicity in ABRAXANE plus gemcitabine-treated patients.

Table 9: Selected Hematologic Laboratory-Detected Abnormalities with a Higher Incidence (≥ 5% for Grades 1-4 or ≥ 2% for Grades 3-4 Events) in the ABRAXANE/Gemcitabine Arm

	ABRAXANE(125 mg/m^2)/ Gemcitabine[d]		Gemcitabine	
	Grades 1-4 (%)	Grade 3-4 (%)	Grades 1-4 (%)	Grade 3-4 (%)
Neutropenia[a,b]	73	38	58	27
Thrombocytopenia[b,c]	74	13	70	9

[a] 405 patients assessed in ABRAXANE/gemcitabine-treated group
[b] 388 patients assessed in gemcitabine-treated group
[c] 404 patients assessed in ABRAXANE/gemcitabine-treated group
[d] Neutrophil growth factors were administered to 26% of patients in the ABRAXANE/gemcitabine group.

Table 10 provides the frequency and severity of adverse reactions which occurred with a difference of ≥ 5% for all grades or ≥ 2% for Grade 3 or higher in the ABRAXANE plus gemcitabine-treated group compared to the gemcitabine group.

Table 10: Selected Adverse Reactions with a Higher Incidence (≥5% for All Grade Toxicity or ≥2% for Grade 3 or Higher Toxicity) in the ABRAXANE/Gemcitabine Arm

System Organ Class	Adverse Reaction	ABRAXANE (125 mg/m^2) and gemcitabine (N=421)		Gemcitabine (N=402)	
		All Grades	Grade 3 or Higher	All Grades	Grade 3 or Higher
General disorders and administration site conditions	Fatigue	248 (59%)	77 (18%)	183 (46%)	37 (9%)
	Peripheral edema	194 (46%)	13 (3%)	122 (30%)	12 (3%)
	Pyrexia	171 (41%)	12 (3%)	114 (28%)	4 (1%)
	Asthenia	79 (19%)	29 (7%)	54 (13%)	17 (4%)
	Mucositis	42 (10%)	6 (1%)	16 (4%)	1 (<1%)
Gastrointestinal disorders	Nausea	228 (54%)	27 (6%)	192 (48%)	14 (3%)
	Diarrhea	184 (44%)	26 (6%)	95 (24%)	6 (1%)
	Vomiting	151 (36%)	25 (6%)	113 (28%)	15 (4%)

System Organ Class	Adverse Reaction	ABRAXANE (125 mg/m^2) and gemcitabine (N=421)		Gemcitabine (N=402)	
		All Grades	Grade 3 or Higher	All Grades	Grade 3 or Higher
Skin and subcutaneous tissue disorders	Alopecia	212 (50%)	6 (1%)	21 (5%)	0
	Rash	128 (30%)	8 (2%)	45 (11%)	2 (<1%)
Nervous system disorders	Peripheral neuropathy[a]	227 (54%)	70 (17%)	51 (13%)	3 (1%)
	Dysgeusia	68 (16%)	0	33 (8%)	0
	Headache	60 (14%)	1 (<1%)	38 (9%)	1 (<1%)
Metabolism and nutrition disorders	Decreased appetite	152 (36%)	23 (5%)	104 (26%)	8 (2%)
	Dehydration	87 (21%)	31 (7%)	45 (11%)	10 (2%)
	Hypokalemia	52 (12%)	18 (4%)	28 (7%)	6 (1%)
Respiratory, thoracic and mediastinal disorders	Cough	72 (17%)	0	30 (7%)	0
	Epistaxis	64 (15%)	1 (<1%)	14 (3%)	1 (<1%)
Infections and infestations	Urinary tract infections[b]	47 (11%)	10 (2%)	20 (5%)	1 (<1%)
Musculoskeletal and connective tissue disorders	Pain in extremity	48 (11%)	3 (1%)	24 (6%)	3 (1%)
	Arthralgia	47 (11%)	3 (1%)	13 (3%)	1 (<1%)
	Myalgia	44 (10%)	4 (1%)	15 (4%)	0
Psychiatric disorders	Depression	51 (12%)	1 (<1%)	24 (6%)	0

[a] Peripheral neuropathy is defined by the MedDRA Version 15.0 Standard MedDRA Query neuropathy (broad scope).
[b] Urinary tract infections includes the preferred terms of: urinary tract infection, cystitis, urosepsis, urinary tract infection bacterial, and urinary tract infection enterococcal.

Additional clinically relevant adverse reactions that were reported in < 10% of the patients with adenocarcinoma of the pancreas who received ABRAXANE/gemcitabine included:

Infections & infestations: oral candidiasis, pneumonia
Vascular disorders: hypertension
Cardiac disorders: tachycardia, congestive cardiac failure
Eye disorders: cystoid macular edema

Peripheral Neuropathy
Grade 3 peripheral neuropathy occurred in 17% of patients who received ABRAXANE/gemcitabine compared to 1% of patients who received gemcitabine only; no patients developed grade 4 peripheral neuropathy. The median time to first occurrence of Grade 3 peripheral neuropathy in the ABRAXANE arm was 140 days. Upon suspension of ABRAXANE dosing, the median time to improvement from Grade 3 peripheral neuropathy to ≤ Grade 1 was 29 days. Of ABRAXANE-treated patients with Grade 3 peripheral neuropathy, 44% resumed ABRAXANE at a reduced dose.

Sepsis
Sepsis occurred in 5% of patients who received ABRAXANE/gemcitabine compared to 2% of patients who received gemcitabine alone. Sepsis occurred both in patients with and without neutropenia. Risk factors for sepsis included biliary obstruction or presence of biliary stent.

Pneumonitis
Pneumonitis occurred in 4% of patients who received ABRAXANE/gemcitabine compared to 1% of patients who received gemcitabine alone. Two of 17 patients in the ABRAXANE arm with pneumonitis died.

6.4 Postmarketing Experience with ABRAXANE and other Paclitaxel Formulations
Unless otherwise noted, the following discussion refers to the adverse reactions that have been identified during post-approval use of ABRAXANE. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure. In some instances, severe events observed with paclitaxel injection may be expected to occur with ABRAXANE.

Hypersensitivity Reactions
Severe and sometimes fatal hypersensitivity reactions have been reported with ABRAXANE. The use of ABRAXANE in patients previously exhibiting hypersensitivity to paclitaxel injection or human albumin has not been studied.

Cardiovascular
There have been reports of congestive heart failure, left ventricular dysfunction, and atrioventricular block with ABRAXANE. Most of the individuals were previously exposed to cardiotoxic drugs, such as anthracyclines, or had underlying cardiac history.

Respiratory
There have been reports of pneumonitis, interstitial pneumonia and pulmonary embolism in patients receiving ABRAXANE and reports of radiation pneumonitis in patients receiving concurrent radiotherapy. Reports of lung fibrosis have been received as part of the continuing surveillance of paclitaxel injection safety and may also be observed with ABRAXANE.

Neurologic
Cranial nerve palsies and vocal cord paresis have been reported, as well as autonomic neuropathy resulting in paralytic ileus.

Vision Disorders
Reports in the literature of abnormal visual evoked potentials in patients treated with paclitaxel injection suggest persistent optic nerve damage. These may also be observed with ABRAXANE.

Reduced visual acuity due to cystoid macular edema (CME) has been reported during treatment with ABRAXANE as well as with other taxanes. After cessation of treatment, CME improves and visual acuity may return to baseline.

Hepatic
Reports of hepatic necrosis and hepatic encephalopathy leading to death have been received as part of the continuing surveillance of paclitaxel injection safety and may occur following ABRAXANE treatment.

Gastrointestinal (GI)
There have been reports of intestinal obstruction, intestinal perforation, pancreatitis, and ischemic colitis following ABRAXANE treatment. There have been reports of neutropenic enterocolitis (typhlitis), despite the coadministration of G-CSF, occurring in patients treated with paclitaxel injection alone and in combination with other chemotherapeutic agents.

Injection Site Reaction
There have been reports of extravasation of ABRAXANE. Given the possibility of extravasation, it is advisable to monitor closely the ABRAXANE infusion site for possible infiltration during drug administration.

Severe events such as phlebitis, cellulitis, induration, necrosis, and fibrosis have been reported as part of the continuing surveillance of paclitaxel injection safety. In some cases the onset of the injection site reaction in paclitaxel injection patients either occurred during a prolonged infusion or was delayed by a week to ten days. Recurrence of skin reactions at a site of previous extravasation following administration of paclitaxel injection at a different site, i.e., "recall", has been reported.

Other Clinical Events
Skin reactions including generalized or maculopapular rash, erythema, and pruritus have been observed with ABRAXANE. There have been case reports of photosensitivity reactions, radiation recall phenomenon, and in some patients previously exposed to capecitabine, reports of palmar-plantar erythrodysesthesia. Stevens-Johnson syndrome and toxic epidermal necrolysis have been reported.

There have been reports of conjunctivitis, cellulitis, and increased lacrimation with paclitaxel injection.

6.5 Accidental Exposure
No reports of accidental exposure to ABRAXANE have been received. However, upon inhalation of paclitaxel, dyspnea, chest pain, burning eyes, sore throat, and nausea have been reported. Following topical exposure, events have included tingling, burning, and redness.

7 DRUG INTERACTIONS
The metabolism of paclitaxel is catalyzed by CYP2C8 and CYP3A4. Caution should be exercised when administering ABRAXANE concomitantly with medicines known to inhibit (e.g., ketoconazole and other imidazole antifungals, erythromycin, fluoxetine, gemfibrozil, cimetidine, ritonavir, saquinavir, indinavir, and nelfinavir) or induce (e.g., rifampicin, carbamazepine, phenytoin, efavirenz, and nevirapine) either CYP2C8 or CYP3A4.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy
Pregnancy Category D *[see Warnings and Precautions (5.8)]*.

There are no adequate and well-controlled studies in pregnant women using ABRAXANE. Based on its mechanism of action and findings in animals, ABRAXANE can cause fetal harm when administered to a pregnant woman. If this drug is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant while receiving ABRAXANE.

Administration of paclitaxel formulated as albumin-bound particles to rats during pregnancy, on gestation days 7 to 17 at doses of 6 mg/m^2 (approximately 2% of the daily maximum recommended human dose on a mg/m^2 basis) caused embryofetal toxicities, as indicated by intrauterine mortality, increased resorptions (up to 5-fold), reduced numbers of litters and live fetuses, reduction in fetal body weight and increase in fetal anomalies. Fetal anomalies included soft tissue and skeletal malformations, such as eye bulge,

folded retina, microphthalmia, and dilation of brain ventricles. A lower incidence of soft tissue and skeletal malformations were also exhibited at 3 mg/m^2 (approximately 1% of the daily maximum recommended human dose on a mg/m^2 basis).

8.3 Nursing Mothers
It is not known whether paclitaxel is excreted in human milk. Paclitaxel and/or its metabolites were excreted into the milk of lactating rats. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants, a decision should be made to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

8.4 Pediatric Use
The safety and effectiveness of ABRAXANE in pediatric patients have not been evaluated.

8.5 Geriatric Use
Of the 229 patients in the randomized study who received ABRAXANE for the treatment of metastatic breast cancer, 13% were at least 65 years of age and < 2% were 75 years or older. No toxicities occurred notably more frequently among patients who received ABRAXANE.

A subsequent pooled analysis was conducted in 981 patients receiving ABRAXANE monotherapy for metastatic breast cancer, of which 15% were 65 years of age or older and 2% were 75 years of age or older. A higher incidence of epistaxis, diarrhea, dehydration, fatigue and peripheral edema was found in patients 65 years of age or older.

Of the 514 patients in the randomized study who received ABRAXANE and carboplatin for the first-line treatment of non-small cell lung cancer, 31% were 65 years or older and 3.5% were 75 years or older. Myelosuppression, peripheral neuropathy, and arthralgia were more frequent in patients 65 years or older compared to patients younger than 65 years old. No overall difference in effectiveness, as measured by response rates, was observed between patients 65 years or older compared to patients younger than 65 years old.

Of the 431 patients in the randomized study who received ABRAXANE and gemcitabine for the first-line treatment of pancreatic adenocarcinoma, 41% were 65 years or older and 10% were 75 years or older. No overall differences in effectiveness were observed between patients who were 65 years of age or older and younger patients. Diarrhea, decreased appetite, dehydration and epistaxis were more frequent in patients 65 years or older compared with patients younger than 65 years old. Clinical studies of ABRAXANE did not include sufficient number of patients with pancreatic cancer who were 75 years and older to determine whether they respond differently from younger patients.

8.6 Patients with Hepatic Impairment
The exposure to paclitaxel may be higher in patients with hepatic impairment than in patients with normal hepatic function. Reduce ABRAXANE starting dose in patients with moderate to severe hepatic impairment. Do not administer ABRAXANE to patients with total bilirubin > 5 x ULN or AST > 10 x ULN *[see Dosage and Administration (2.4), Warnings and Precautions (5.6) and Clinical Pharmacology (12.3)]*. Do not administer to patients with metastatic adenocarcinoma of the pancreas who have moderate to severe hepatic impairment *[see Dosage and Administration (2.4)]*.

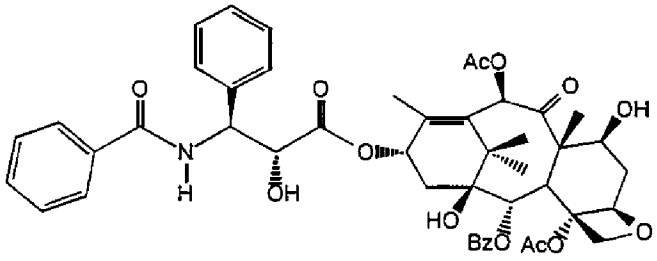
8.7 Patients with Renal Impairment
Adjustment of the starting ABRAXANE dose is not required for patients with mild to moderate renal impairment (estimated creatinine clearance ≥30 to <90 mL/min) *[see Clinical Pharmacology (12.3)]*. There are insufficient data to permit dosage recommendations in patients with severe renal impairment or end stage renal disease (estimated creatinine clearance <30 mL/min).

10 OVERDOSAGE
There is no known antidote for ABRAXANE overdosage. The primary anticipated complications of overdosage would consist of bone marrow suppression, sensory neurotoxicity, and mucositis.

11 DESCRIPTION
ABRAXANE for Injectable Suspension (paclitaxel protein-bound particles for injectable suspension) (albumin-bound) is paclitaxel formulated as albumin-bound nanoparticles with a mean particle size of approximately 130 nanometers. Paclitaxel exists in the particles in a non-crystalline, amorphous state. ABRAXANE is supplied as a white to yellow, sterile, lyophilized powder for reconstitution with 20 mL of 0.9% Sodium Chloride Injection, USP prior to intravenous infusion. Each single-use vial contains 100 mg of paclitaxel (bound to human albumin) and approximately 900 mg of human albumin (containing sodium caprylate and sodium acetyltryptophanate). Each milliliter (mL) of reconstituted suspension contains 5 mg paclitaxel formulated as albumin-bound particles. ABRAXANE is free of solvents.

The active agent in ABRAXANE is paclitaxel, a microtubule inhibitor. The chemical name for paclitaxel is 5β,20-Epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine.

Paclitaxel has the following structural formula:



Paclitaxel is a white to off-white crystalline powder with the empirical formula $C_{47}H_{51}NO_{14}$ and a molecular weight of 853.91. It is highly lipophilic, insoluble in water, and melts at approximately 216°C to 217°C.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

ABRAXANE is a microtubule inhibitor that promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. This stability results in the inhibition of the normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic cellular functions. Paclitaxel induces abnormal arrays or "bundles" of microtubules throughout the cell cycle and multiple asters of microtubules during mitosis.

12.3 Pharmacokinetics

Absorption

The pharmacokinetics of total paclitaxel following 30 and 180-minute infusions of ABRAXANE at dose levels of 80 to 375 mg/m^2 were determined in clinical studies. Dose levels of mg/m^2 refer to mg of paclitaxel in ABRAXANE. Following intravenous administration of ABRAXANE, paclitaxel plasma concentrations declined in a biphasic manner, the initial rapid decline representing distribution to the peripheral compartment and the slower second phase representing drug elimination.

The drug exposure (AUCs) was dose proportional over 80 to 300 mg/m^2 and the pharmacokinetics of paclitaxel for ABRAXANE were independent of the duration of intravenous administration.

The pharmacokinetic data of 260 mg/m^2 ABRAXANE administered over a 30-minute infusion was compared to the pharmacokinetics of 175 mg/m^2 paclitaxel injection over a 3-hour infusion. Clearance was larger (43%) and the volume of distribution was higher (53%) for ABRAXANE than for paclitaxel injection. There were no differences in terminal half-lives.

Distribution

Following ABRAXANE administration to patients with solid tumors, paclitaxel is evenly distributed into blood cells and plasma and is highly bound to plasma proteins (94%). In a within-patient comparison study, the fraction of unbound paclitaxel in plasma was significantly higher with ABRAXANE (6.2%) than with solvent-based paclitaxel (2.3%). This contributes to significantly higher exposure to unbound paclitaxel with ABRAXANE compared with solvent-based paclitaxel, when the total exposure is comparable. *In vitro* studies of binding to human serum proteins, using paclitaxel concentrations ranging from 0.1 to 50 µg/mL, indicated that the presence of cimetidine, ranitidine, dexamethasone, or diphenhydramine did not affect protein binding of paclitaxel. The total volume of distribution is approximately 1741 L; the large volume of distribution indicates extensive extravascular distribution and/or tissue binding of paclitaxel.

Metabolism

In vitro studies with human liver microsomes and tissue slices showed that paclitaxel was metabolized primarily to 6α-hydroxypaclitaxel by CYP2C8; and to two minor metabolites, 3'-*p*-hydroxypaclitaxel and 6α, 3'-*p*-dihydroxypaclitaxel, by CYP3A4. *In vitro*, the metabolism of paclitaxel to 6α-hydroxypaclitaxel was inhibited by a number of agents (ketoconazole, verapamil, diazepam, quinidine, dexamethasone, cyclosporin, teniposide, etoposide, and vincristine), but the concentrations used exceeded those found *in vivo* following normal therapeutic doses. Testosterone, 17α-ethinyl estradiol, retinoic acid, and quercetin, a specific inhibitor of CYP2C8, also inhibited the formation of 6α-hydroxypaclitaxel *in vitro*. The pharmacokinetics of paclitaxel may also be altered *in vivo* as a result of interactions with compounds that are substrates, inducers, or inhibitors of CYP2C8 and/or CYP3A4 *[see Drug Interactions (7)]*.

Elimination

At the clinical dose range of 80 to 300 mg/m^2, the mean total clearance of paclitaxel ranges from 13 to 30 L/h/m^2, and the mean terminal half-life ranges from 13 to 27 hours.

After a 30-minute infusion of 260 mg/m^2 doses of ABRAXANE, the mean values for cumulative urinary recovery of unchanged drug (4%) indicated extensive non-renal clearance. Less than 1% of the total administered dose was excreted in urine as the metabolites 6α-hydroxypaclitaxel and 3'-*p*-hydroxypaclitaxel.

Fecal excretion was approximately 20% of the total dose administered.

Specific Populations

Pharmacokinetics in Hepatic Impairment
The effect of hepatic impairment on the pharmacokinetics of paclitaxel following ABRAXANE administration was studied in patients with advanced solid tumors. The results showed that mild hepatic impairment (total bilirubin >1 to ≤1.5 x ULN, AST ≤10 x ULN, n=8) had no clinically important effect on pharmacokinetics of paclitaxel. Patients with moderate (total bilirubin >1.5 to ≤ 3 x ULN, AST ≤10 x ULN, n=7) or severe (total bilirubin >3 to ≤5 x ULN, n=5) hepatic impairment had a 22% to 26% decrease in the maximum elimination rate of paclitaxel and approximately 20% increase in mean paclitaxel AUC compared with patients with normal hepatic function (total bilirubin ≤ULN, AST ≤ULN, n=130). *[see Dosage and Administration (2.4) and Use in Specific Populations (8.6)]*.

Elimination of paclitaxel shows an inverse correlation with total bilirubin and a positive correlation with serum albumin. Pharmacokinetic/pharmacodynamic modeling indicates that there is no correlation between hepatic function (as indicated by the baseline albumin or total bilirubin level) and neutropenia after adjusting for ABRAXANE exposure. Pharmacokinetic data are not available for patients with total bilirubin >5 x ULN or for patients with metastatic adenocarcinoma of the pancreas *[see Dosage and Administration (2.4) and Use in Specific Populations (8.6)]*.

Pharmacokinetics in Renal Impairment
The effect of pre-existing mild (creatinine clearance ≥60 to <90 mL/min, n=61) or moderate (creatinine clearance ≥30 to <60 mL/min, n=23) renal impairment on the pharmacokinetics of paclitaxel following ABRAXANE administration was studied in patients with advanced solid tumors. Mild to moderate renal impairment had no clinically important effect on the maximum elimination rate and systemic exposure (AUC and C_{max}) of paclitaxel *[see Use in Specific Populations (8.7)]*.

Other Intrinsic Factors
Population pharmacokinetic analyses for ABRAXANE show that body weight (40 to 143 kg), body surface area (1.3 to 2.4 m^2), gender, race (Asian vs. White), age (24 to 85 years) and type of solid tumors do not have a clinically important effect on the maximum elimination rate and systemic exposure (AUC and C_{max}) of paclitaxel.

Pharmacokinetic Interactions between ABRAXANE and Carboplatin
Administration of carboplatin immediately after the completion of the ABRAXANE infusion to patients with NSCLC did not cause clinically meaningful changes in paclitaxel exposure. The observed mean AUC_{inf} of free carboplatin was approximately 23% higher than the targeted value (6 min*mg/mL), but its mean half-life and clearance were consistent with those reported in the absence of paclitaxel.

Pharmacokinetic Interactions between ABRAXANE and Gemcitabine
Pharmacokinetic interactions between ABRAXANE and gemcitabine have not been studied in humans.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility
The carcinogenic potential of ABRAXANE has not been studied.

Paclitaxel was clastogenic *in vitro* (chromosome aberrations in human lymphocytes) and *in vivo* (micronucleus test in mice). ABRAXANE was not mutagenic in the Ames test or the CHO/HGPRT gene mutation assay.

Administration of paclitaxel formulated as albumin-bound particles to male rats at 42 mg/m^2 on a weekly basis (approximately 16% of the daily maximum recommended human exposure on a body surface area basis) for 11 weeks prior to mating with untreated female rats resulted in significantly reduced fertility accompanied by decreased pregnancy rates and increased loss of embryos in mated females. A low incidence of skeletal and soft tissue fetal anomalies was also observed at doses of 3 and 12 mg/m^2/week in this study (approximately 1 to 5% of the daily maximum recommended human exposure on a mg/m^2 basis). Testicular atrophy/degeneration was observed in single-dose toxicology studies in rodents administered paclitaxel formulated as albumin-bound particles at doses lower than the recommended human dose; doses were 54 mg/m^2 in rodents and 175 mg/m^2 in dogs.

14 CLINICAL STUDIES

14.1 Metastatic Breast Cancer
Data from 106 patients accrued in two single arm open label studies and from 460 patients enrolled in a randomized comparative study were available to support the use of ABRAXANE in metastatic breast cancer.

Single Arm Open Label Studies
In one study, ABRAXANE was administered as a 30-minute infusion at a dose of 175 mg/m^2 to 43 patients with metastatic breast cancer. The second trial utilized a dose of 300 mg/m^2 as a 30-minute infusion in 63 patients with metastatic breast cancer. Cycles were administered at 3-week intervals. Objective responses were observed in both studies.

Randomized Comparative Study
This multicenter trial was conducted in 460 patients with metastatic breast cancer. Patients were randomized to receive ABRAXANE at a dose of 260 mg/m^2 given as a 30-minute infusion, or paclitaxel injection at 175 mg/m^2 given as a 3-hour infusion. Sixty-four percent of patients had impaired performance status (ECOG 1 or 2) at study entry; 79% had visceral metastases; and 76% had > 3 sites of metastases. Fourteen percent of the patients had not received prior chemotherapy; 27% had received chemotherapy in the adjuvant setting, 40% in the metastatic setting and 19% in both metastatic and adjuvant settings. Fifty-nine

percent received study drug as second or greater than second-line therapy. Seventy-seven percent of the patients had been previously exposed to anthracyclines.

In this trial, patients in the ABRAXANE treatment arm had a statistically significantly higher reconciled target lesion response rate (the trial primary endpoint) of 21.5% (95% CI: 16.2% to 26.7%), compared to 11.1% (95% CI: 6.9% to 15.1%) for patients in the paclitaxel injection treatment arm. See Table 11. There was no statistically significant difference in overall survival between the two study arms.

Table 11: Efficacy Results from Randomized Metastatic Breast Cancer Trial

		ABRAXANE 260 mg/m^2	Paclitaxel Injection 175 mg/m^2
Reconciled Target Lesion Response Rate (primary endpoint)[a]			
All randomized patients	Response Rate [95% CI]	50/233 (21.5%) [16.19% – 26.73%]	25/227 (11.1%) [6.94% – 15.09%]
	p-value[b]	0.003	
Patients who had failed combination chemotherapy or relapsed within 6 months of adjuvant chemotherapy[c]	Response Rate [95% CI]	20/129 (15.5%) [9.26% – 21.75%]	12/143 (8.4%) [3.85% – 12.94%]

[a] Reconciled Target Lesion Response Rate (TLRR) was the prospectively defined protocol specific endpoint, based on independent radiologic assessment of tumor responses reconciled with investigator responses (which also included clinical information) for the first 6 cycles of therapy. The reconciled TLRR was lower than the investigator Reported Response Rates, which are based on all cycles of therapy.

[b] From Cochran-Mantel-Haenszel test stratified by 1st line vs. > 1st line therapy.

[c] Prior therapy included an anthracycline unless clinically contraindicated.

14.2 Non-Small Cell Lung Cancer

A multicenter, randomized, open-label study was conducted in 1052 chemonaive patients with Stage IIIb/IV non-small cell lung cancer to compare ABRAXANE in combination with carboplatin to paclitaxel injection in combination with carboplatin as first-line treatment in patients with advanced non-small cell lung cancer. ABRAXANE was administered as an intravenous infusion over 30 minutes at a dose of 100 mg/m^2 on Days 1, 8, and 15 of each 21-day cycle. Paclitaxel injection was administered as an intravenous infusion over 3 hours at a dose of 200 mg/m^2, following premedication. In both treatment arms carboplatin at a dose of AUC = 6 mg•min/mL was administered intravenously on Day 1 of each 21-day cycle after completion of ABRAXANE/paclitaxel infusion. Treatment was administered until disease progression or development of an unacceptable toxicity. The major efficacy outcome measure was overall response rate as determined by a central independent review committee using RECIST guidelines (Version 1.0).

In the intent-to-treat (all-randomized) population, the median age was 60 years, 75% were men, 81% were White, 49% had adenocarcinoma, 43% had squamous cell lung cancer, 76% were ECOG PS 1, and 73% were current or former smokers. Patients received a median of 6 cycles of treatment in both study arms.

Patients in the ABRAXANE/carboplatin arm had a statistically significantly higher overall response rate compared to patients in the paclitaxel injection/carboplatin arm [(33% versus 25%) see Table 12]. There was no statistically significant difference in overall survival between the two study arms.

Table 12: Efficacy Results from Randomized Non-Small Cell Lung Cancer Trial (Intent-to-Treat Population)

	ABRAXANE (100 mg/m^2 weekly) + carboplatin (N=521)	Paclitaxel Injection (200 mg/m^2 every 3 weeks) + carboplatin (N=531)
Overall Response Rate (ORR)		
Confirmed complete or partial overall response, n (%)	170 (33%)	132 (25%)
95% CI	28.6, 36.7	21.2, 28.5
P-value (Chi-Square test)	0.005	
Median DoR in months (95% CI)	6.9 (5.6, 8.0)	6.0 (5.6, 7.1)
Overall Response Rate by Histology		
Carcinoma/Adenocarcinoma	66/254 (26%)	71/264 (27%)
Squamous Cell Carcinoma	94/229 (41%)	54/221 (24%)
Large Cell Carcinoma	3/9 (33%)	2/13 (15%)
Other	7/29 (24%)	5/33 (15%)

CI = confidence interval; DoR= Duration of response

14.3 Adenocarcinoma of the Pancreas

A multicenter, multinational, randomized, open-label study was conducted in 861 patients comparing ABRAXANE plus gemcitabine versus gemcitabine monotherapy as first-line treatment of metastatic adenocarcinoma of the pancreas. Key eligibility criteria were Karnofsky Performance Status (KPS) ≥70, normal bilirubin level, transaminase levels ≤ 2.5 times the upper limit of normal (ULN) or ≤ 5 times the ULN for patients with liver metastasis, no prior cytotoxic chemotherapy in the adjuvant setting or for metastatic disease, no ongoing active infection requiring systemic therapy, and no history of interstitial lung disease. Patients with rapid decline in KPS (≥10%) or serum albumin (≥20%) during the 14 day screening period prior to study randomization were ineligible.

A total of 861 patients were randomized (1:1) to the ABRAXANE/gemcitabine arm (N=431) or to the gemcitabine arm (N=430). Randomization was stratified by geographic region (Australia, Western Europe, Eastern Europe, or North America), KPS (70 to 80 versus 90 to 100), and presence of liver metastasis (yes versus no). Patients randomized to ABRAXANE/gemcitabine received ABRAXANE 125 mg/m^2 as an intravenous infusion over 30-40 minutes followed by gemcitabine 1000 mg/m^2 as an intravenous infusion over 30-40 minutes on Days 1, 8, and 15 of each 28-day cycle. Patients randomized to gemcitabine received 1000 mg/m^2 as an intravenous infusion over 30-40 minutes weekly for 7 weeks followed by a 1-week rest period in Cycle 1 then as 1000 mg/m^2 on Days 1, 8 and 15 of each subsequent 28-day cycle. Patients in both arms received treatment until disease progression or unacceptable toxicity. The major efficacy outcome measure was overall survival (OS). Additional outcome measures were progression-free survival (PFS) and overall response rate (ORR), both assessed by independent, central, blinded radiological review using RECIST (version 1.0).

In the intent to treat (all randomized) population, the median age was 63 years (range 27-88 years) with 42% ≥ 65 years of age; 58% were men; 93% were White and KPS was 90-100 in 60%. Disease characteristics included 46% of patients with 3 or more metastatic sites; 84% of patients had liver metastasis; and the location of the primary pancreatic lesion was in the head of pancreas (43%), body (31%), or tail (25%).

Results for overall survival, progression-free survival, and overall response rate are shown in Table 13.

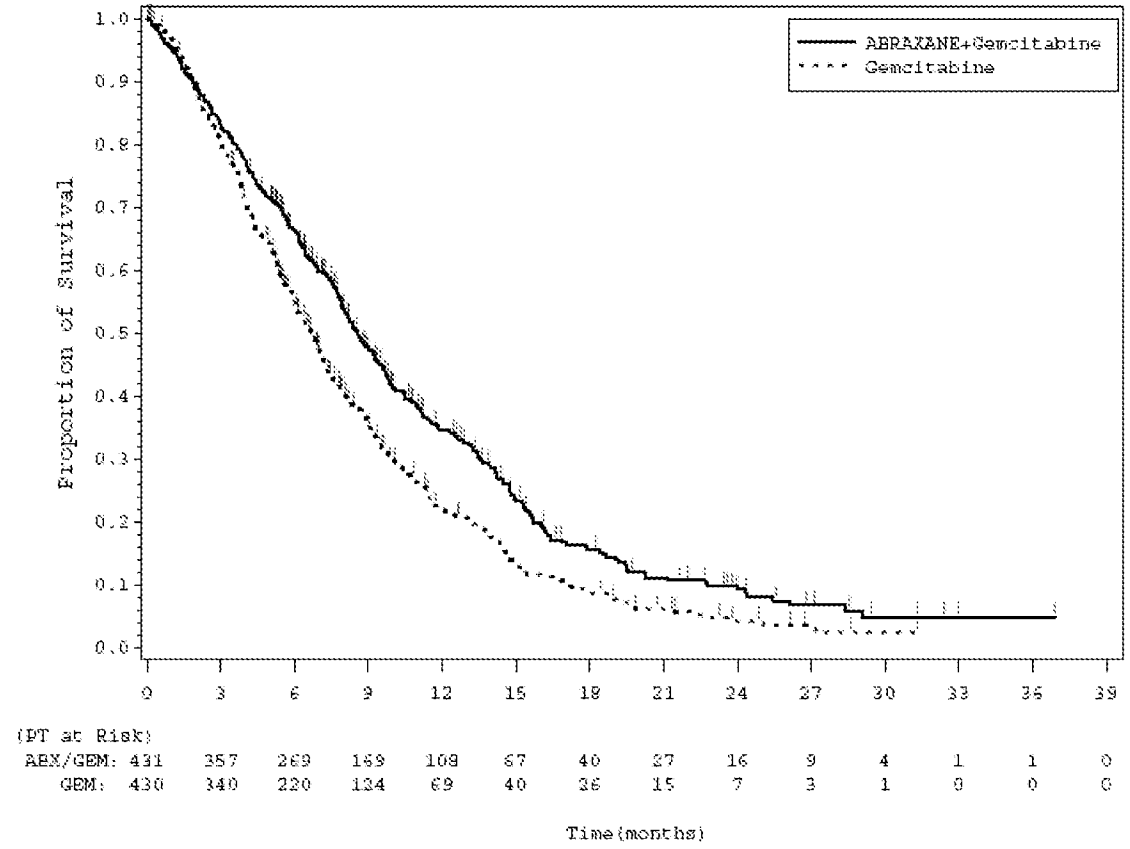
Table 13: Efficacy Results from Randomized Study in Patients with Adenocarcinoma of the Pancreas (ITT Population)

	ABRAXANE(125 mg/m^2) and gemcitabine (N = 431)	**Gemcitabine (N = 430)**
Overall Survival		
Number of deaths, n (%)	333 (77)	359 (83)
Median Overall Survival (months)	**8.5**	**6.7**
95% CI	7.9, 9.5	6.0, 7.2
HR (95% CI) [a]	0.72 (0.62, 0.83)	
P-value [b]	<0.0001	
Progression-free Survival [c]		
Death or progression, n (%)	277 (64)	265 (62)
Median Progression-free Survival (months)	**5.5**	**3.7**
95% CI	4.5, 5.9	3.6, 4.0
HR (95% CI) [a]	0.69 (0.58, 0.82)	
P-value [b]	<0.0001	
Overall Response Rate [c]		
Confirmed complete or partial overall response, n (%)	**99 (23)**	**31 (7)**
95% CI	19.1, 27.2	5.0, 10.1
P-value [d]	<0.0001	

CI = confidence interval, HR = hazard ratio of ABRAXANE plus gemcitabine / gemcitabine, ITT = intent-to-treat population.
[a] Stratified Cox proportional hazard model.
[b] Stratified log-rank test stratified by geographic region (North America versus Others), Karnofsky performance score (70 to 80 versus 90 to 100), and presence of liver metastasis (yes versus no).
[c] Based on Independent Radiological Reviewer Assessment.
[d] Chi-square test.

In exploratory analyses conducted in clinically relevant subgroups with a sufficient number of subjects, the treatment effects on overall survival were similar to that observed in the overall study population.

Figure 1: Kaplan-Meier Curve of Overall Survival (Intent-to-treat Population)



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3. American Society of Health-System Pharmacists. (2006) ASHP Guidelines on Handling Hazardous Drugs. *Am J Health-Syst Pharm.* 2006;63:1172-1193.

4. Polovich, M., White, J. M., & Kelleher, L.O. (eds.) 2005. Chemotherapy and biotherapy guidelines and recommendations for practice (2nd. ed.) Pittsburgh, PA: Oncology Nursing Society.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

Product No.: 103450
NDC No.: 68817-134-50 100 mg of paclitaxel in a single-use vial, individually packaged in a carton.

16.2 Storage

Store the vials in original cartons at 20°C to 25°C (68°F to 77°F). Retain in the original package to protect from bright light.

16.3 Handling and Disposal

Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published *[see References (15)]*. There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate.

17 PATIENT COUNSELING INFORMATION

See FDA-approved patient labeling

- ABRAXANE injection may cause fetal harm. Advise patients to avoid becoming pregnant while receiving this drug. Women of childbearing potential should use effective contraceptives while receiving ABRAXANE *[see Warnings and Precautions (5.8) and Use in Specific Populations (8.1)]*.
- Advise men not to father a child while receiving ABRAXANE *[see Warnings and Precautions (5.9)]*.
- Patients must be informed of the risk of low blood cell counts and severe and life-threatening infections and instructed to contact their physician immediately for fever or evidence of infection. *[see Warnings and Precautions (5.1), (5.3)]*.
- Patients should be instructed to contact their physician for persistent vomiting, diarrhea, or signs of dehydration.
- Patients must be informed that sensory neuropathy occurs frequently with ABRAXANE and patients should advise their physicians of numbness, tingling, pain or weakness involving the extremities *[see Warnings and Precautions (5.2)]*.
- Explain to patients that alopecia, fatigue/asthenia, and myalgia/arthralgia occur frequently with ABRAXANE
- Instruct patients to contact their physician for signs of an allergic reaction, which could be severe and sometimes fatal. *[see Warnings and Precautions (5.5)]*.
- Instruct patients to contact their physician immediately for sudden onset of dry persistent cough, or shortness of breath *[see Warnings and Precautions (5.4)]*.

Manufactured for: Celgene Corporation
Summit, NJ 07901

U.S. Patent Numbers: See www.celgene.com.

ABRPI.009/PPI.009 07/15

Patient Information

ABRAXANE® (ah-BRAKS-ane)
(paclitaxel protein-bound particles for injectable suspension)
(albumin-bound)

Read this Patient Information before you start receiving ABRAXANE and before each infusion. This information does not take the place of talking with your doctor about your medical condition or your treatment.

What is ABRAXANE?

ABRAXANE is a prescription medicine used to treat:

- advanced breast cancer in people who have already received certain other medicines for their cancer.
- advanced non-small cell lung cancer, in combination with carboplatin in people who cannot be treated with surgery or radiation.
- and advanced pancreatic cancer, when used in combination with gemcitabine as the first medicine for advanced pancreatic cancer.

It is not known if ABRAXANE is safe or effective in children.

Who should not receive ABRAXANE?

Do not receive ABRAXANE if:

- your white blood cell count is below 1,500 cells/ mm^3.
- you have had a severe allergic reaction to ABRAXANE.

What should I tell my doctor before receiving ABRAXANE?

Before you receive ABRAXANE, tell your doctor if you:

- have liver or kidney problems.
- have any other medical conditions.
- are a man planning to father a child. You should not father a child during your treatment with ABRAXANE. ABRAXANE can harm the unborn baby of your partner. Talk to your doctor if this is a concern to you.
- are pregnant or plan to become pregnant. ABRAXANE can harm your unborn baby. You should not become pregnant while receiving ABRAXANE. Women who may become pregnant should use effective birth control (contraception). Talk to your doctor about the best way to prevent pregnancy while receiving ABRAXANE.
- are breastfeeding or plan to breastfeed. It is not known if ABRAXANE passes into your breast milk. You and your doctor should decide if you will receive ABRAXANE or breastfeed.

Tell your doctor about all the medicines you take, including prescription and over-the-counter medicines, vitamins, and herbal supplements.

Know the medicines you take. Keep a list to show your doctor and pharmacist when you get a new medicine.

How will I receive ABRAXANE?

- Your doctor will prescribe ABRAXANE in an amount that is right for you.
- Premedication to prevent allergic reactions is generally not needed to receive ABRAXANE. Premedication may be needed if you have had an allergic reaction to ABRAXANE. In case of severe allergic reaction, ABRAXANE should not be used again.
- ABRAXANE will be given to you by intravenous infusion into your vein.
- Your doctor should do regular blood tests while you receive ABRAXANE.

What are the possible side effects of ABRAXANE?

ABRAXANE may cause serious side effects, including:

- decreased blood cell counts. ABRAXANE can cause a severe decrease in neutrophils (a type of white blood cells important in fighting against bacterial infections) and platelets (important for clotting and to control bleeding). Your doctor will check your blood cell count during your treatment with ABRAXANE and after you have stopped your treatment.
- numbness, tingling, pain, or weakness in your hands or feet (neuropathy).
- severe infection (sepsis). If you receive ABRAXANE in combination with gemcitabine, infections can be severe and lead to death. Tell your doctor right away if you have a fever (temperature of greater than 100.4° F) or develop signs of infection.
- lung or breathing problems. If you receive ABRAXANE in combination with gemcitabine, lung or breathing problems may be severe and can lead to death. Tell your doctor right away if you have a sudden onset of persistent dry cough or shortness of breath.
- allergic reactions. Allergic reactions to ABRAXANE may be severe and can lead to death.

The most common side effects of ABRAXANE include:

- hair loss
- numbness, tingling, pain, or weakness in the hands or feet
- abnormal heart beat
- tiredness
- joint and muscle pain
- changes in your liver function tests
- rash
- low red blood cell count (anemia). Red blood cells carry oxygen to your body tissues. Tell your doctor if you feel weak, tired or short of breath.
- nausea and vomiting
- infections. If you have a fever (temperature of greater than 100.4º F) or other signs of infection, tell your doctor right away.

- Diarrhea
- Loss of body fluid (dehydration)
- Swelling in the hands or feet

These are not all the possible side effects of ABRAXANE. For more information, ask your doctor or pharmacist.

Call your doctor for medical advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

General information about the safe and effective use of ABRAXANE.

Medicines are sometimes prescribed for purposes other than those listed in a Patient Information leaflet.

This Patient Information leaflet summarizes the important information about ABRAXANE. If you would like more information, talk to your doctor. You can ask your doctor or pharmacist for information about ABRAXANE that is written for health professionals.

For more information, call 1-888-423-5436.

What are the ingredients in ABRAXANE?

Active ingredient: paclitaxel (bound to human albumin).

Other ingredient: human albumin (containing sodium caprylate and sodium acetyltryptophanate).

This Patient Information has been approved by the U.S. Food and Drug Administration.

Revised: July 2015

Manufactured for: Celgene Corporation
Summit, NJ 07901

ABRAXANE® is a registered trademark of Abraxis BioScience, LLC.

Abraxis BioScience, LLC is a wholly owned subsidiary of Celgene Corporation.

U.S. Patent Numbers: See www.celgene.com.

ABRPPI.009 07/15

[CANCER RESEARCH 53, 1484–1488, April 1, 1993]

Advances In Brief

Antibody-targeted Delivery of Doxorubicin Entrapped in Sterically Stabilized Liposomes Can Eradicate Lung Cancer in Mice[1]

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Abstract

Cancer chemotherapy is limited by adverse side effects resulting from toxicities to normal tissues. Targeted delivery of drugs to diseased tissues *in vivo* would help to reduce these side effects. Liposomes containing lipid derivatives of polyethylene glycol have circulation times sufficiently long to allow for effective *in vivo* drug delivery. Polyethylene glycol liposomes, containing entrapped doxorubicin, targeted to KLN-205 squamous cell carcinoma of the lung by means of specific antibodies attached at the liposome surface were capable of reducing tumor burden to a high degree and eradicating tumor in a significant percentage of mice.

Introduction

The therapeutic efficacy of anticancer drugs is restricted by dose-limiting toxicities against normal tissues *in vivo*. Selective toxicity to tumor cells of anticancer drugs, *e.g.*, DOX,[3] would be greatly improved if the drugs could be directed to the tumor cells and away from other sensitive tissues such as heart and bone marrow. The concept of drug targeting was introduced at the turn of the century by Paul Ehrlich (1) after the discovery of antibodies. Two decades ago, phospholipid bilayer spheres, called liposomes, were first postulated to be good candidates for site-specific delivery of drugs (2). However, formulations of liposomes used in the past were recognized as foreign particles *in vivo*, which resulted in their rapid removal from circulation by the cells of the MPS. The sequestration of liposomes within the MPS caused damage to this important host defense system and prevented the liposomes from reaching other sites of action (reviewed in Ref. 3). Drug targeting became more feasible with the recent development of new formulations of liposomes, containing either monosialoganglioside GM_1 or lipid derivatives of polyethylene glycol, termed Stealth (Liposome Technology, Inc., Menlo Park, CA) or S-liposomes, which avoided MPS uptake thereby resulting in long circulation half-lives and dose-independent pharmacokinetics (4–10). We have recently demonstrated antibody-mediated specific binding and selective cytotoxicity of liposome-entrapped DOX to lung cancer cells *in vitro* (11). We now describe, for the first time ever, targeting and selective toxicity toward neoplastic cells of liposome-entrapped drugs *in vivo*.

A mAb, which selectively recognizes a unique epitope on proteins expressed on the proliferating cells of mammalian squamous carcinomas (12), has been developed for diagnostic use in humans. This IgG_1 mAb, 174H.64, also binds to epitopes on a murine lung squamous carcinoma, KLN-205, that lodges in the lung in DBA mice within 3 days following i.v. injection (12, 13). It has been demonstrated that mAb 174H.64 selectively stains the highly proliferative peripheral stem cell layer of KLN-205 lung tumors as well as those of human and bovine squamous carcinomas (12, 13). Since these stem cells are the progenitors of the more differentiated cells in the interior of the tumor which may no longer be capable of proliferation (14, 15), targeting antineoplastic drugs selectively to the proliferating stem cell population may be of therapeutic value.

Materials and Methods

Materials. IgG_1 mAb 174H.64 directed against mammalian squamous carcinoma was a generous gift of Biomira, Inc. (Edmonton, Alberta, Canada). Polyethylene glycol covalently linked to PEG-DSPE and HSPC was the generous gift of Liposome Technology, Inc. All other materials were as previously described (11).

Liposome Preparation. Liposomal DOX and mAb liposomal DOX were prepared as described in Ref. 11. Briefly, liposomes, were composed of HSPC:CH:PEG-DSPE, 2:1:0.1 molar ratio, extruded to an average diameter of 112 nm (range, 98 to 116 nm), and were injected at a phospholipid dose of 1.2–1.4 μmol/mouse and a mAb 174H.64 dose, where applicable, of 10.5–12.5 μg/mouse. 174H.64 mAb was biotinylated and attached to the surface of S-liposomes containing biotinylated phosphatidylethanolamine by means of an avidin linker (11). This procedure did not change the size of the liposomes or result in liposome aggregation. DOX was encapsulated by a remote loading method previously described (16).

Deoxyuridine Uptake and *in Vivo* Survival Experiments. DBA/2 mice (Jackson Laboratories) were given i.v. injections of 2×10^5 KLN-205 cells. At 3 days postinjection of KLN-205 cells, the treatment groups were given single i.v. injections of either 0.2 ml phosphate-buffered saline (untreated controls) or 6 mg/kg of either free DOX, DOX entrapped in HSPC:CH:PEG-DSPE liposomes (liposomal DOX), or DOX entrapped in HSPC:CH:PEG-DSPE liposomes containing attached antibody 174H.64 (mAb liposomal DOX), all in 0.2 ml of sterile saline. Some mice received mAb liposomes (11–39 μg mAb) lacking DOX. On the 45th day after injection, mice were given injections of 2 μCi each of [^{125}I]dUrd and 4 h later the lungs were removed, cut into small pieces, washed with 10% trichloroacetic acid, and counted in a gamma counter. For *in vivo* survival experiments the injection protocols were identical to the above, except the survival of the mice was monitored daily until evidence of morbidity resulted in their termination, at which point gross pathological examination of their internal organs for the presence of tumor nodules was performed.

Histopathology. Mice given injections of 2×10^5 KLN-205 cells and 3 days postinjection of tumor they were treated with single i.v. injections of 6 mg/kg free DOX, liposomal DOX, or mAb liposomal DOX as in the previous section. On day 45, lungs were removed from the different treatment groups. Tumour masses were randomly located throughout all lung fields and midsagittal sections were made from each of the largest lung lobes. The right and left caudal lobes were fixed in 10% neutral buffered formalin, processed into paraffin blocks, sectioned at 5 μm, and stained with hematoxylin and eosin.

Liposome Uptake into Lung. Uptake of liposomes labeled with [^{125}I]-tyraminylinulin into lung in DBA/2 mice (3/group) was measured at 15 min postinjection because we have previously shown that uptake of mAb liposomes by KLN-205 cells *in vitro* was very rapid (11). Uptake experiments, which provide a measure of uptake of intact liposomes, were performed as detailed in Ref. 7, including correction for tissue blood volume. Liposome uptake was

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[1] This work was supported by the Medical Research Council of Canada (MA-9127).

[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: DOX, doxorubicin; mAb, IgG_1 murine monoclonal antibody(s) 174H.64; MPS, mononuclear phagocyte system; HSPC, hydrogenated soy phosphatidylcholine; CH, cholesterol; PEG-DSPE, polyethylene glycol (M_r 1900) covalently coupled to distearoylphosphatidylethanolamine; MST, mean survival time; S-liposomes, sterically stabilized or Stealth liposomes; [^{125}I]dUrd, [^{125}I]iododeoxyuridine.

examined at 3 and 45 days after i.v. injection of 2×10^5 KLN-205 cells. Experimental groups were composed of tumor-free (control) animals receiving either HSPC:CH:PEG-DSPE or mAb HSPC:CH:PEG-DSPE liposomes (2:1:0.1 molar ratio, no drug), or tumor-bearing animals receiving similar liposomes. Results are reported as cpm/mg lung tissue, normalized to 10^6 injected cpm of [^{125}I]tyraminylinulin.

Results and Discussion

[^{125}I]dUrd uptake experiments were performed in mice as a measure of the effect of the various drug treatments on KLN-205 tumor cell proliferation (Fig. 1). Uptake into lung of [^{125}I]dUrd has been established to be directly proportional to the number of i.v. injected KLN-205 tumor cells in the range of 10^5 to 10^6 injected cells (13). Mice receiving 2×10^5 KLN-205 cells were examined for [^{125}I]dUrd uptake at 45 days postinjection. Negligible levels of [^{125}I]dUrd were found in the lungs of tumor-free mice (normal controls). Untreated tumor-bearing mice (positive controls) had significantly increased levels of uridine uptake ($P < 0.001$), as would be expected in the presence of proliferating tumor cells. All drug treatment groups resulted in significant decreases in [^{125}I]dUrd uptake in the order of: mAb liposomal DOX > liposomal DOX > free DOX > untreated controls ($P < 0.01$ to 0.001). Treatment of tumor-bearing mice with single i.v. injections of mAb liposomal DOX resulted in an 80% reduction in the uptake of [^{125}I]dUrd. The levels of [^{125}I]dUrd uptake in this group of mice were not statistically different from those found in normal mice, suggesting that the tumor burden had been very substantially reduced. Gross pathological inspection of the lungs was made for all animals. Those receiving the mAb liposomal DOX had apparently normal lungs with no evidence of tumor nodules. The numbers of tumor nodules in the lungs of mice receiving liposomal DOX appeared to be decreased compared to mice receiving free DOX or no treatment.

Histopathology slides were prepared at 45 days postinjection from the various treatment groups in Fig. 2 and compared to slides prepared from tumor-free mice and mean tumor diameters were determined (Table 1). No differences in nodule number or mean diameter were observed in mice receiving free DOX (6 mg/kg) compared to untreated mice (Fig. 2, *B versus C*; Table 1). A decrease in nodule number was found for mice receiving liposomal DOX (Fig. 2*D*; Table 1). A dramatic decrease in the number of tumor nodules was observed in the mice receiving a single i.v. injection of 6 mg/kg DOX entrapped in mAb liposomes (Fig. 2, *E* and *F*; Table 1). Histopathology was performed in two of five animals receiving mAb liposomes, none of which had any apparent tumor nodules on gross inspection. In one animal no tumor foci were observed (Fig. 2*E*), while in the other animal only two tumor foci were observed in the right lung (Fig. 2*F*; Table 1) and one foci was in the left (Table 1). The tumors in the mice treated with mAb liposomal DOX appeared smaller and more dense and had a necrotic appearance compared to the tumors seen in the other treatment groups (Fig. 2F). These results confirmed the [^{125}I]-dUrd uptake results, providing direct evidence of the high degree of efficacy of the mAb-targeted liposomal DOX.

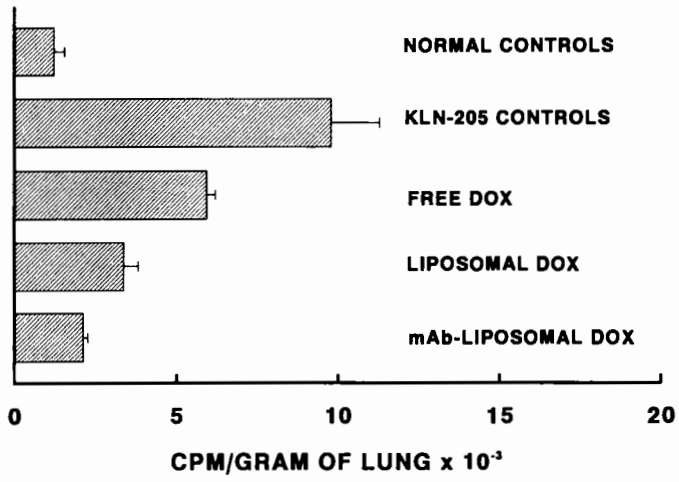
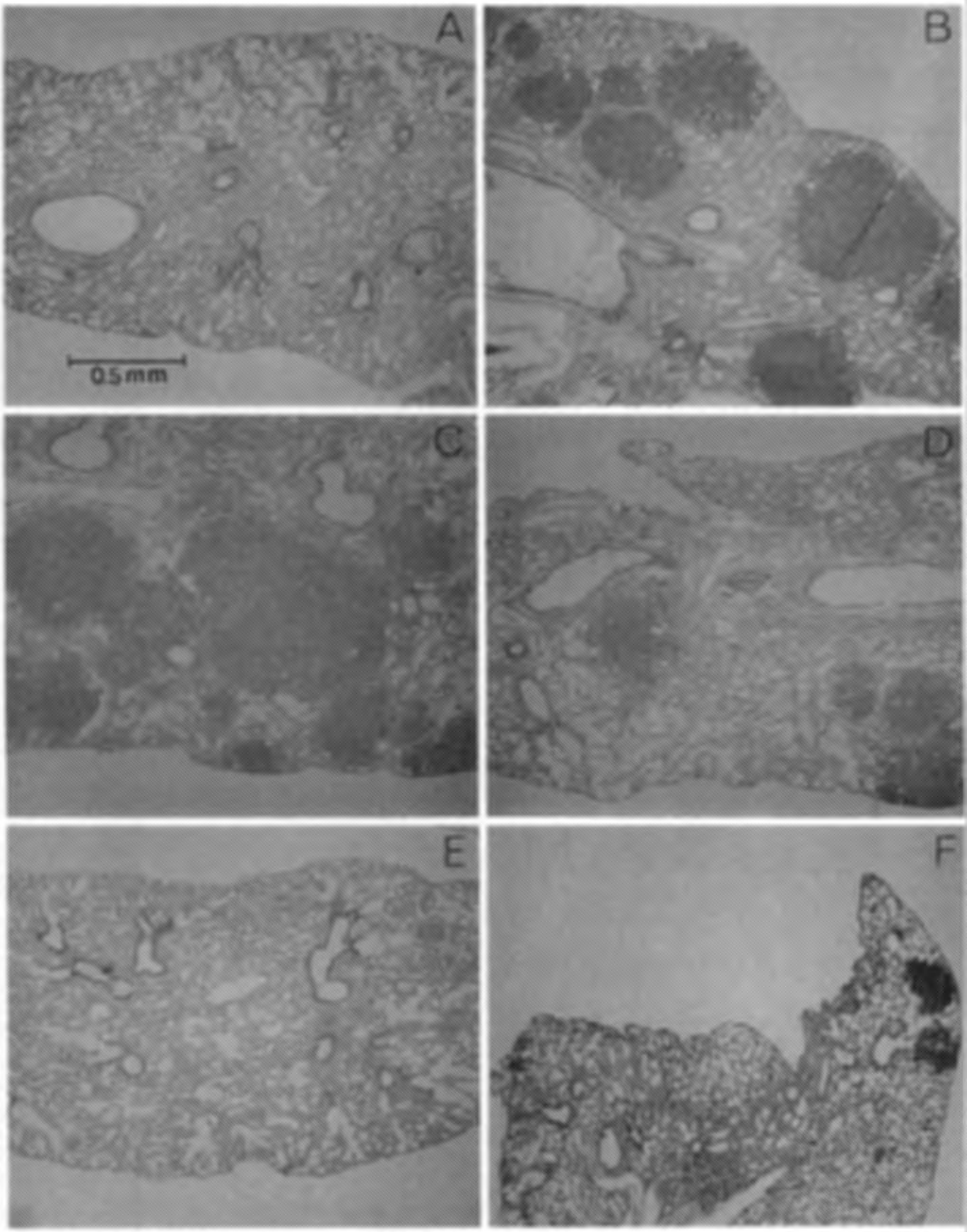


Fig. 1. ^{125}dUrd uptake in normal and tumor-bearing DBA/2 mice (3/group) 45 days after i.v. injection of 2×10^5 KLN-205 cells suspended in 0.2 ml phosphate-buffered saline. Three days postinjection mice were treated with 6 mg/kg of either free DOX, liposomal DOX, or mAb liposomal DOX. Liposomes were composed of HSPC:CH:PEG-DSPE, 2:1:0.1 molar ratio (average diameter, 112 nm), and mice received 1.2–1.4 μmol phospholipid and 10.5–12.5 μg mAb.

In an initial survival experiment, in which mice were given a single injection of 2 mg/kg DOX in mAb liposomes 3 days postinjection of tumor, efficacy of the liposomal preparation *in vivo* was demonstrated, with treated mice having significantly increased mean survival times ($P < 0.001$; MST ± SD) averaging 90.7 ± 4.6 days ($n = 3$, excluding 2 sacrificed mice; see below) compared to 64.8 ± 5.1 for untreated mice ($n = 5$). Two mice in the treatment group were terminated on days 102 and 157, respectively, with no sign of disease in their lungs upon gross pathological inspection. Fig. 3 shows the survival data for mice (2×5/group) receiving various treatments at DOX doses of 6 mg/kg, which is still well below the maximum tolerated dose for liposomal DOX, which in our hands is >18 mg/kg for mAb liposomal DOX (data not shown). The dose that is lethal to 50% of the population for free DOX is approximately 23 mg/kg and liposome encapsulation of DOX in the presence or absence of PEG decreases drug toxicity (8, 17), the degree of toxicity reduction depending on liposome composition (17). The MST ± SD of the mice treated with mAb liposomal DOX that died of their tumors (99.2 ± 36.1 days, exclusive of long-term survivors) was significantly greater ($P < 0.001$) than for untreated mice (52.0 ± 5.8) or for mice treated with either free DOX (MST of 57.6 ± 9.3) or liposomal DOX (MST of 65.8 ± 13.1). Two mice in the mAb liposomal DOX group were sacrificed on day 170 with no evidence of tumor; the other three surviving mice continue to be monitored. Treatment of mice with empty liposomes (no drug; no mAb) at the same lipid dose had no therapeutic effect (data not shown). Although free mAb given at doses of 300 μg/mouse × 5 (1.5 mg total) resulted in a modest increase in MST in mice (13), mAb liposomes (no drug) given at mAb doses up to 3 times those used in our experiments (11–39 μg mAb/mouse or 40–130-fold lower level of mAb) had no therapeutic effect (Fig. 3). Nonspecific antibody-drug conjugates have been previously shown to result in no growth inhibition of KLN-205 cells *in vivo* and no increase in survival times in mice (13), nor do they mediate binding of liposomes to KLN-205 cells *in vitro* (11); therefore, controls involving nonspecific antibodies were not done in this series of experiments.

When mAb were linked to S-liposomes they retained their long circulation half-lives. Twenty-four h postinjection, 34.7 ± 6.7% of *in vivo* mAb liposomes containing PEG-DSPE were in blood ($T_{½}$ = 16.2 h) for normal, tumor-free mice ($n = 6$), similar to the levels for S-liposomes in the absence of antibody (37.5 ± 9.7% at 24 h, $t_{½}$ = 19.0 h). These results can be compared to the undetectable blood levels (<1%) for mAb liposomes in the absence of PEG-DSPE at 24 h postinjection ($t_{½\alpha}$ = 0.6 h; $t_{½\beta}$ = 4.0 h). A description of the pharmacokinetics of mAb liposomes in the presence and absence of PEG-DSPE is in work.[4] Blood levels of mAb liposomes dropped to 22.8 ± 1.9% at 24 h in tumor-bearing (45-day) mice, which is likely a consequence of increased tumor uptake (see below). Uptake of liposomes, labeled with the aqueous space label [^{125}I]tyraminylinulin,

[4] Manuscript in preparation.



DOX, liposomal DOX, or mAb liposomal DOX as outlined in Fig. 1. *A*, normal lung; *B*, untreated; *C*, free DOX; *D*, liposomal DOX; *E* and *F*, mAb liposomal DOX. Bar, 0.5 mm.

Table 1 *Number and diameter of discrete tumor nodules/linear cm of lung right and left caudal lobes in midsagittal sections stained with hematoxylin and eosin*

The lungs of all animal within each experimental group appear similar upon gross inspection and measurements were made for 1 typical animal from each group of 5 animals examined, except in the case of mAb liposomal DOX in which numbers were determined from 2 typical animals

	Left lung		Right lung	
Treatment group	No. of foci	Diameter (mm ± SD)	No. of foci	Diameter (mm ± SD)
Normal (control)	0		0	
Untreated (control)	28	0.43 ± 0.18	34	0.41 ± 0.19
Free DOX	40	0.43 ± 0.18	23	0.36 ± 0.21
Liposomal DOX	10	0.31 ± 0.06	19	0.39 ± 0.23
mAb-liposomal DOX	0.3	0.22	0.7	0.18, 0.26

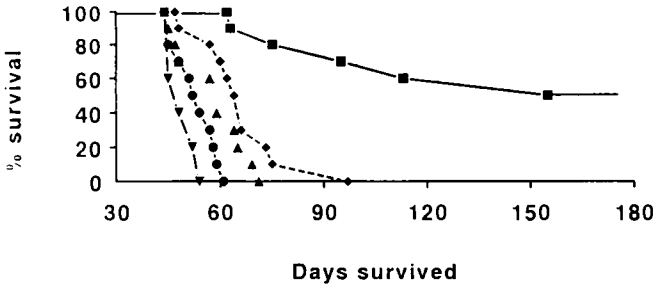


Fig. 3. DBA/2 mice (20–25 g; (2 × 5/group) were inoculated i.v. with 2 × 10^5 KLN-205 cells suspended in 0.2 ml phosphate-buffered saline. Treatment was initiated on the third day with 6 mg/kg of either free DOX (▲), liposomal DOX (◆), mAb liposomal DOX (■), or mAb liposomes (▼) (12 μg mAb, no DOX) as outlined in Fig. 1. Control groups (●) were treated sterile phosphate-buffered saline. Survival times of the mice were noted, and gross pathology was done on all animals. Two long-term survivors were sacrificed on day 170 and showed no evidence of tumor; the remaining animals continue to be monitored.

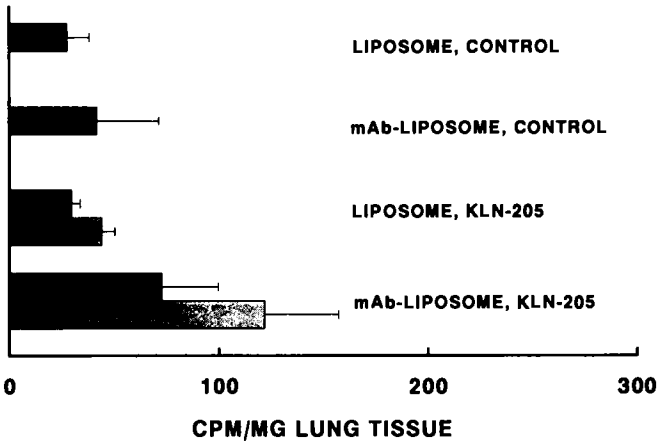


Fig. 4. Uptake of liposomes labeled with [^{125}I]tyraminylinulin into lung in DBA/2 mice (3/group) at 15 min postinjection. Uptake experiments were performed as detailed in Ref. 8. Liposome uptake was examined at 3 days (■) and 45 days (▨) after i.v. injection of 2 × 10^5 KLN-205 cells. Test groups were composed of tumor-free (control) or tumor-bearing (KLN-205) mice receiving either HSPC:CH:PEG-DSPE (2:1:0.1) liposomes or mAb liposomes of the same composition.

at 15 min postinjection, into the lungs of untreated (receiving no drug) mice was examined at 3 and 45 days postinjection of tumor (Fig. 4). Three days postinjection there was a marginally significant increase ($P < 0.05$) in uptake of mAb liposomes into the lungs of tumor-bearing lung at 15 min postinjection of liposomes, as compared to tumor-free mice, while a significant increase ($P < 0.01$) in lung uptake of liposomes was observed at 45 days postinjection. At 2 and 24 h postinjection, cpm/mg of mAb liposomes in tumor-bearing lungs were approximately double those in tumor-free lungs (data not shown). Lung uptake at 45 days postinjection of tumor was 5.0 ± 1.1% of *in vivo* mAb liposomes for tumor-bearing mice compared to 1.3 ± 0.2% for tumor-free mice at 24 h postinjection of liposomes. We did not expect high levels of lung uptake, since relatively small numbers of proliferating cells would be present in mice at both 3 and 45 days postinjection of tumor and the numbers of stem cells would be only a tiny percentage of total lung tissue in both cases. At 45 days postinjection, while there was a large tumor burden, the majority of tumor was composed of differentiated cells which do not bind to the mAb (12, 13). The increased uptake of mAb liposomes into tumor-bearing lungs suggests that they are able to bind to proliferating tumor cells localized in the lung.

The results obtained from several different approaches, *i.e.*, [^{125}I]-dUrd uptake, tissue pathology, tissue distribution and survival data, combine to demonstrate that *in vivo* targeting of antineoplastic drugs entrapped in S-liposomes can be achieved, with excellent therapeutic efficacy. Maruyama *et al.* (18) have previously shown increased targeting of long-circulating liposomes containing monosialoganglioside GM_1, as compared to conventional liposomes, to lung by means of an antibody against lung endothelial cells attached to the liposome surface. Our experiments are the first demonstration of *in vivo* targeting of liposome-entrapped anticancer drugs resulting in enhanced therapeutic efficacy in a tumor model. It is notable that eradication of tumor could be obtained in several mice using single injections of liposomes targeted by means of low levels of mAb and containing very modest levels of DOX. This suggests that increasing the dose of DOX in the mAb liposomes or giving multiple injections of these liposomes could substantially increase our ability to eradicate tumor in mice. These experiments are currently underway. Longenecker *et al.* (13) have demonstrated the efficacy of mAb 174H.64-daunomycin conjugates in extending the survival times of mice bearing KLN-205 tumor, although 300 μg mAb were needed to deliver 10 μg drug to individual mice. In the current study we found that a reduction in mAb:drug ratios of 400-fold could be achieved, with approximately 11 μg of mAb required to deliver 150 μg DOX to individual mice (6 mg/kg DOX). An additional advantage of targeted liposomes over antibody-drug conjugates or immunotoxins might occur in the presence of tumor heterogeneity. Specific binding of a drug package to a tumor cell, with release and diffusion of the drug and uptake by surrounding tumor cells, may allow killing of cells which lack the specific epitope, since cytotoxicity of liposome-entrapped DOX, at least *in vitro*, is mediated by the release of drug from the liposomes and uptake of free drug by tumor cells (19).

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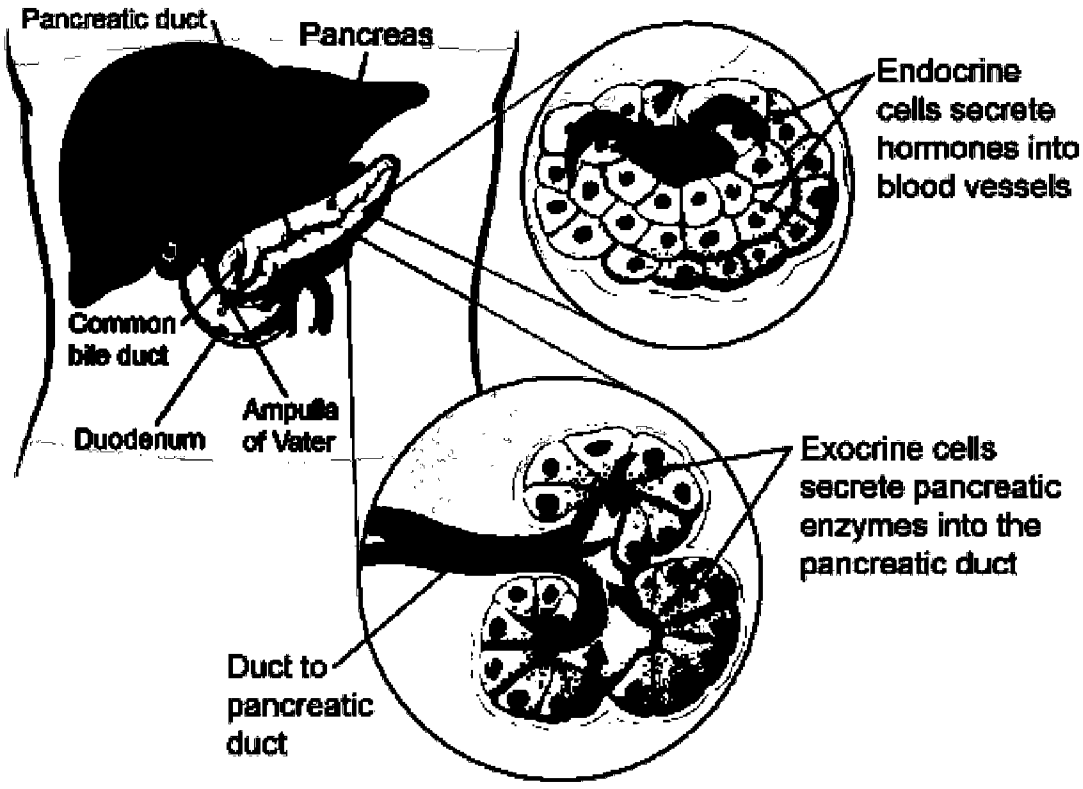
What Is Pancreatic Cancer?

Cancer starts when cells in the body begin to grow out of control. Cells in nearly any part of the body can become cancer, and can spread to other areas of the body. See What Is Cancer? to learn more about how cancers start and spread.

Pancreatic cancer starts when cells in the pancreas start to grow out of control. The pancreas is an organ that sits behind the stomach. It's shaped a bit like a fish with a wide head, a tapering body, and a narrow, pointed tail. In adults it's about 6 inches long but less than 2 inches wide. The head of the pancreas is on the right side of the abdomen (belly), behind where the stomach meets the duodenum (the first part of the small intestine). The body of the pancreas is behind the stomach, and the tail of the pancreas is on the left side of the abdomen next to the spleen.



The pancreas has 2 main types of cells:

- **Exocrine cells:** Most of the cells in the pancreas form the exocrine glands and ducts. The exocrine glands make pancreatic enzymes that are released into the intestines to help you digest foods (especially fats). The enzymes are first released into tiny tubes called *ducts*. These merge to form larger ducts, which empty into the pancreatic duct. The pancreatic duct merges with the common bile duct (the duct that carries bile from the liver), and empties into the duodenum (the first part of the small intestine) at the ampulla of Vater.
- **Endocrine cells:** Endocrine cells make up a much smaller percentage of the cells in the pancreas. These cells are in small clusters called *islets* (or *islets of Langerhans*). These islets make important hormones like insulin and glucagon (which help control blood sugar levels), and release them directly into the blood.

Types of pancreatic cancer

The exocrine cells and endocrine cells of the pancreas form different types of tumors. It's very important to know if the cancer in the pancreas is an exocrine or endocrine cancer. They have distinct risk factors and causes, have different signs and symptoms, are diagnosed with different tests, are treated in different ways, and have different outlooks.

Exocrine pancreatic cancers

Exocrine cancers are by far the most common type of pancreas cancer. If you are told you have pancreatic cancer, it's most likely an exocrine pancreatic cancer.

Pancreatic adenocarcinoma: About 95% of cancers of the exocrine pancreas are adenocarcinomas. These cancers usually start in the ducts of the pancreas. Less often, they develop from the cells that make the pancreatic enzymes, in which case they are called *acinar cell carcinomas*.

Less common types of exocrine cancer: Other, less common exocrine cancers include adenosquamous carcinomas, squamous cell carcinomas, signet ring cell carcinomas, undifferentiated carcinomas, and undifferentiated carcinomas with giant cells.

Ampullary cancer (carcinoma of the ampulla of Vater): This cancer starts in the ampulla of Vater, which is where the bile duct and pancreatic duct come together and empty into the small intestine. Ampullary cancers aren't technically pancreatic cancers, but they are included here because they are treated much the same.

Ampullary cancers often block the bile duct while they're still small and have not spread far. This blockage causes bile to build up in the body, which leads to yellowing of the skin and eyes (jaundice). Because of this, these cancers are usually found earlier than most pancreatic cancers, and they usually have a better prognosis (outlook).

Pancreatic endocrine tumors (neuroendocrine tumors)

Tumors of the endocrine pancreas are uncommon, making up less than 5% of all pancreatic cancers. As a group, they are often called *pancreatic neuroendocrine tumors (NETs)* or *islet cell tumors*.

Pancreatic NETs can be benign (not cancer) or malignant (cancer). Benign and malignant tumors can look alike under a microscope, so it isn't always clear if a pancreatic NET is really cancer. Sometimes it only becomes clear that an NET is cancer when it spreads outside the pancreas.

There are many types of pancreatic NETs.

Functioning NETs: About half of pancreatic NETs make hormones that are released into the blood and cause symptoms. These are called *functioning* tumors. Each one is named for the

type of hormone the tumor cells make.

- **Gastrinomas** come from cells that make gastrin. About half of gastrinomas are cancers.
- **Insulinomas** come from cells that make insulin. Most insulinomas are benign (not cancer).
- **Glucagonomas** come from cells that make glucagon. Most glucagonomas are cancers.
- **Somatostatinomas** come from cells that make somatostatin. Most somatostatinomas are cancers.
- **VIPomas** come from cells that make vasoactive intestinal peptide (VIP). Most VIPomas are cancers.
- **PPomas** come from cells that make pancreatic polypeptide. Most PPomas are cancers.

Most functioning NETs are gastrinomas or insulinomas. The other types are rare.

Non-functioning NETs: These tumors don't make enough excess hormones to cause symptoms. They are more likely to be cancer than are functioning tumors. Because they don't make excess hormones that cause symptoms, they can often grow quite large before they're found.

Carcinoid tumors: These NETs are much more common in other parts of the digestive system, although rarely they can start in the pancreas. These tumors often make serotonin (also called *5-HT*) or its precursor, 5-HTP.

The treatment and outlook for pancreatic NETs depend on the specific tumor type and the stage (extent) of the tumor, but the outlook is generally better than that of pancreatic exocrine cancers.

Benign and precancerous growths in the pancreas

Some growths in the pancreas are simply benign (not cancer), while others might become cancer over time if left untreated (known as *precancers*). Because people are getting imaging tests such as CT scans more often than in the past (for a number of reasons), these types of pancreatic growths are now being found more often.

Serous cystic neoplasms (SCNs) (also known as *serous cystadenomas*) are tumors that have sacs (cysts) filled with watery fluid. SCNs are almost always benign, and most don't

need to be treated unless they grow large or cause symptoms.

Mucinous cystic neoplasms (MCNs) (also known as *mucinous cystadenomas*) are slow-growing tumors that have cysts filled with a jelly-like substance called *mucin*. These tumors almost always occur in women. While they are not cancer, some of them can progress to cancer over time if not treated, so these tumors are typically removed with surgery.

Intraductal papillary mucinous neoplasms (IPMNs) are benign tumors that grow in the pancreatic ducts. Like MCNs, these tumors make mucin, and over time they sometimes become cancer if not treated. Some IPMNs can just be followed closely over time, but some might need to be removed with surgery if they have certain features, such as if they are in the main pancreatic duct.

Solid pseudopapillary neoplasms (SPNs) are rare, slow-growing tumors that almost always develop in young women. Even though these tumors tend to grow slowly, they can sometimes spread to other parts of the body, so they are best treated with surgery. The outlook for people with these tumors is usually very good.

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From antinutrient to phytonutrient: phytic acid gains respect.(Ask EN)

Magazine
Environmental Nutrition
April 1, 2004

Q. I've heard that phytic acid interferes with the body's ability to absorb important minerals. Now I see it sold as a supplement in the health food store. Is it one and the same?

A. Yes. Phytate or phytic acid, also known as inositol hexaphosphate or IP6 (the name used by one supplement manufacturer), has long been considered a nutrition "bad guy" because it latches on to minerals such as iron, zinc and calcium, reducing their absorption. Phytate is bound to the fiber in plant foods, including whole grains, legumes, nuts and seeds.

However, new research from animal and test tube experiments has shown that phytate also functions as an antioxidant and may actually play more of a protective role in health.

Can IP6 Combat Cancer? Most of the research on IP6 has focused on its potential role in cancer prevention and treatment. In laboratory experiments, IP6 appears to have a tumor-blocking effect on many types of human cells including breast, lung, colon and prostate, explains Ivana Vucenik, Ph.D., of the University of Maryland School of Medicine in Baltimore. IP6 appears to work on cancer cells by normalizing cell growth and increasing apoptosis (cell suicide), says Vucenik.

A Helping Hand With Heart Disease?

IP6 may provide similar protection against heart disease, explains Harvard's Simin Liu, Ph.D., head researcher of the Nurses' Health Study. He found that women who ate two to three serving a day of whole grains had about 30% less risk of heart disease compared to those who ate less than half a serving a day.

"There are many possible mechanisms that might be responsible for the beneficial effects seen in our study," he says, "one of which may certainly include phytates."

Mineral Loss a Cause for Concern? Phytic acid's mineral-binding action may be less of a concern, especially for calcium, than previously believed. "While phytates have a strong inhibitory effect on iron and zinc, their effect on calcium is small," says Connie Weaver, Ph.D., of Purdue University, who has conducted research in this area. Just be sure you get enough of all three minerals in your diet or from a multi, she advises.

EN's Bottom Line. It's premature to rush out and buy IP6 supplements. Instead, aim for 20 to 35 grams of fiber from a variety of sources, including fruits, vegetables, whole grains, nuts and seeds, every day. EN will keep you posted as we learn more.

Irinophore C, a Novel Nanoformulation of Irinotecan, Alters Tumor Vascular Function and Enhances the Distribution of 5-Fluorouracil and Doxorubicin

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Abstract

Purpose: To examine the antitumor effects of Irinophore C, a nanopharmaceutical formulation of irinotecan, on the tissue morphology and function of tumor vasculature in HT-29 human colorectal tumors.

Experimental Design: Fluorescence microscopy was used to map and quantify changes in tissue density, tumor vasculature, hypoxia, and the distribution of Hoechst 33342, a perfusion marker, and the anticancer drug, doxorubicin. Noninvasive magnetic resonance imaging was used to quantify K_{trans}, the volume transfer constant of a solute between the blood vessels and extracellular tissue compartment of the tumor, as a measure of vascular function. Following treatment with Irinophore C, ^{19}F magnetic resonance spectroscopy was used to monitor the delivery of 5-fluorouracil (5-FU) to the tumor tissue, whereas scintigraphy was used to quantify the presence of bound [^{14}C]5-FU.

Results: Irinophore C decreased cell density ($P = 8.42 \times 10^{-5}$), the overall number of endothelial cells in the entire section ($P = 0.014$), tumor hypoxia ($P = 5.32 \times 10^{-9}$), and K_{trans} ($P = 0.050$). However, treatment increased the ratio of endothelial cells to cell density ($P = 0.00024$) and the accumulation of Hoechst 33342 ($P = 0.022$), doxorubicin ($P = 0.243 \times 10^{-5}$), and 5-FU ($P = 0.0002$) in the tumor. Vascular endothelial growth factor and interleukin-8, two proangiogenic factors, were down-regulated, whereas the antiangiogenic factor TIMP-1 was up-regulated in Irinophore C-treated tumors.

Conclusions: Irinophore C treatment improves the vascular function of the tumor, thereby reducing tumor hypoxia and increasing the delivery and accumulation of a second drug. Reducing hypoxia would enhance radiotherapy, whereas improving delivery of a second drug to the tumor should result in higher cell kill.

The clinical management of metastatic disease originating from colon/colorectal cancer remains challenging. The liver is the most common site of distant metastases for colorectal cancer, with 70% of patients presenting with liver metastases followed by the lungs, bone, and brain (1, 2). At present, the only cure is complete surgical removal of the primary tumor if diagnosed early; however, up to 45% of these patients still relapse with metastatic disease. Standard of care for first-line therapy in patients is a combination of 5-fluorouracil (5-FU; plus leucovorin) with either irinotecan (FOLFIRI) or oxaliplatin (FOLFOX; ref. 3). The treatments are associated with prolonged median survivals of 18 to 21 months. Capecitabine, an oral fluoropyrimidine carbamate, has also been used in combination with 5-FU, and clinical data suggest that this combination is comparable with the FOLFIRI and FOLFOX regimens (4, 5). In practice, however, combination therapy with capecitabine is limited because of severe toxicities such as hand-foot syndrome, diarrhea, nausea, vomiting, and bone marrow suppression (4, 5). More recently, monoclonal antibodies targeting the epidermal growth factor receptor, such as cetuximab and panitumumab, have been used in combination with standard chemotherapy with promising results (6). The safety and efficacy of bevacizumab, the monoclonal antibody that targets vascular endothelial growth factor (VEGF; ref. 7), in combination with FOLFIRI or FOLFOX, was also evaluated recently (8, 9). Although both studies were carried out with small

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Translational Relevance

Median survival times of 18 to 21 months are associated with irinotecan-based treatments for metastatic colorectal cancer. We report here a novel formulation of irinotecan, Irinophore C, which improves oxygenation levels and the delivery and accumulation of a second drug in a tumor model for colorectal cancer. Increasing oxygen levels would potentiate radiotherapy, and improving delivery of a second drug should increase cell kill. If human tumors respond similarly to Irinophore C, replacing irinotecan with Irinophore C in current combination therapies could improve the delivery of the partner agents (oxaliplatin, 5-FU, or leucovorin). Imaging techniques (magnetic resonance imaging and positron emission tomography) could be used to monitor changes in the tumor and help delineate the use of sequential therapies to target specific treatment induced changes in the tumor microenvironment (e.g., radiotherapy when oxygen levels improve). Integrating Irinophore C into clinical use should be relatively easy because irinotecan is clinically approved for colorectal cancer.

patient numbers, Kang et al. concluded that the combination treatment had modest activity and was relatively tolerable in metastatic colorectal cancer that had failed FOLFIRI or FOLFOX. A retrospective study where bevacizumab and FOLFOX were used in first-line treatment of metastatic colorectal cancer (8) further indicated that the treatment improved time to disease progression and overall survival. The authors concluded that this combination should be explored further. Finally, there is interest in using radiotherapy concurrently with chemotherapy in an adjuvant setting (10, 11).

Of particular interest to our group, the positive results with the antiangiogenic activity of bevacizumab indicate that the vasculature of colorectal tumors is an important therapeutic target in metastatic colorectal cancer. The clinical utility of combination therapies underlines the fact that novel approaches targeting more than one of the hallmarks of aggressive cancer should be explored more fully. More specifically, there is interest in targeting tumor vasculature and cancer cells in combination. Thus, for example, vascular disrupting agents have been used as single agents to shut down blood vessels in tumors and also in combination with chemotherapy (12, 13). The utility of antiangiogenic agents in clinical trials as single agents and in combination with chemoradiation is also being examined (14, 15).

Although newer treatments have improved the outcome of metastatic colorectal cancer from 6 months without treatment to 2 years with treatment, we believe that further improvements in survival time are achievable using innovative therapeutics and carefully designed treatment scheduling. An attractive proposition is to devise a sequence of therapies designed to take advantage of treatment-induced changes in the tumor microenvironment that can be readily measured using noninvasive imaging methods. We recently reported on the improved efficacy of a novel lipid-based formulation of irinotecan (Irinophore C) where treatment outcomes in 5 xenograft models, including the HT-29 and LS-180 models for colorectal cancer, were significantly improved (16, 17). In brief, the difference in pharmacokinetic profiles between free Irinotecan (Camptosar) and Irinophore C is best emphasized by the 1,000-fold increase in the plasma area-under-the-curve of the lactone form of irinotecan, the active form of the drug, seen with Irinophore C compared with that of Camptosar. Irinotecan is also metabolized by carboxylesterase to yield SN-38, which is 100- to 1,000-fold more potent *in vitro* than the parent drug. Like irinotecan, SN-38 also exists in equilibrium as the lactone and carboxylate forms, and we have also shown that both forms of SN-38 were detected in the plasma 5 min after injection of Camptosar. In contrast, only the lactone form was detected in the plasma following Irinophore C administration. The plasma levels of SN-38 lactone peaked at 2 μg/mL within 1 h, decreased to 1 μg/mL after 4 h, and remained constant thereafter over 24 h; however, the plasma concentrations of SN-38 at 24 h were still greater than those observed for Camptosar 10 min after administration.

To gain a better understanding of the mechanism behind the antitumor activity of Irinophore C, we examined the treatment-induced effects on the tumor microenvironment in the HT-29 colorectal cancer model. Our interest in assessing the tumor microenvironment was driven in part by publications suggesting that irinotecan inhibits angiogenesis (18) and also by the potential for systemically administered nanopharmaceuticals to achieve antivascular effects comparable with those achieved with metronomic dosing (19). The studies described here used multimodality imaging methods to assess tumor-associated vascular structure and function. These include noninvasive magnetic resonance imaging/spectroscopy and tumor mapping of biological markers in tumor sections. Fluorescence microscopy and tumor mapping techniques were used to quantify the observed decreases in tumor blood vessel content, hypoxia, and viable cell density. Treatment with Irinophore C increased the accumulation of a perfusion marker (Hoechst 33342). Magnetic resonance imaging indicated that vascular function improved following treatment with Irinophore C. The vascular changes were further associated with enhanced delivery of doxorubicin and 5-FU determined with fluorescence microscopy and ^{19}F magnetic resonance spectroscopy (MRS), respectively.

Materials and Methods

Tumor model. Animal studies were approved by the University of British Columbia Animal Care Committee and conducted in accordance with guidelines from the Canadian Council for Animal Care. The HT-29 tumor model for colon cancer was used in these studies; 5×10^6 cells (50 μL medium) were injected subcutaneously into the lower backs of female Rag2M mice. Tumors appeared within 2 weeks following cell inoculation and mice were randomly separated into 7 groups at this time (6 mice per group, unless indicated otherwise). Treatments were initiated when tumors reached an average volume of ~150 mm^3 (~0.5 cm in diameter). Tumor volume was calculated as volume = 0.5 length (cm) × width $(cm)^2$. Dimensions were measured using calipers by the same technician throughout the duration of the studies.

Treatment groups and drug/marker administration. Procedures for encapsulating irinotecan in liposomes have been described previously (16). In brief, irinotecan was encapsulated in 1,2-distearoyl-*sn*-glycerophosphocholine and cholesterol distearoyl phosphatidylcholine/cholestrol at a molar ratio of 55:45. The distearoyl phosphatidylcholine/

cholestrol/cholesterol lipid films formed were hydrated at 65°C in a solution of 300 mmol/L copper sulfate solution and subjected to 5 cycles of freeze-and-thaw. The multilamellar vesicle suspensions were extruded 10 times through polycarbonate filters with defined pore size to obtain unilamellar vesicles with a mean size distribution of 100 to 140 nm. The external buffer of the liposomes was then exchanged using Sephadex G-50 size exclusion chromatography, with HEPES-buffered solution containing EDTA (pH 7.5). The liposomes were then preincubated in the presence of the divalent metal ionophore A23187 at 60°C for 30 min. Subsequently, irinotecan hydrochloride trihydrate was added to liposomes at 50°C in a drug-to-lipid ratio of 0.2:1 (mol/mol); under these conditions, >98% of the added drug associates with the liposomes. These drug-loaded liposomes are then exchanged into a non-EDTA-containing buffer and adjusted to an irinotecan concentration suitable for administration to mice. It should be noted that a scaled version of this formulation process has been developed for batch sizes of 1 L; stability studies to date suggest that the drug-loaded preparation is stable for >6 months at 4°C. The formulation was administered (25 mg/kg, active drug ingredient) to one group once a week for 3 weeks and the tumors allowed to regrow after treatment was stopped. The remaining 6 groups of mice received either saline or Irinophore C once a week for 6 weeks with a 10-day break after week 3 of the treatment regimen. The tumors in one group (and saline control group) were used to assess tumor growth delay, K_{trans} values, and levels of hypoxia. The hypoxia marker EF5 (30 mg/kg; ref. 20) and the fluorescent dye Hoechst 33342 (16 mg/kg; Sigma) were injected intravenously into mice 180 and 20 min before sacrifice, respectively. Tumors were harvested and portions were cryopreserved in tissue preservative (OCT) or flash-frozen in liquid nitrogen for subsequent molecular analysis. The remaining two groups (and controls) were used to assess the tumor accumulation of 5-FU and doxorubicin (Sigma-Aldrich). A single bolus injection of 5-FU [200 mg/kg, labeled with [^{14}C]5-FU (0.6 μCi/mL)] was administered intravenously via a catheter placed into the animal's lateral tail vein for ^{19}F MRS scans. One hour after scans were completed, the mice were euthanized and the tumors were harvested for scintillation counting. Doxorubicin (30 mg/kg) was injected intravenously 40 min before harvesting tumors that were cryopreserved (OCT) in liquid N_2 vapor. A portion of tumors from the group used to examine the distribution of 5-FU with MRS was also fixed in formalin and embedded in paraffin blocks.

Immunohistochemistry. Tumor cryosections (10 or 20 μm, as indicated) were cut using a Cryostar HM560 (Microm International), air-dried, and imaged for exogenous marker native fluorescence (Hoechst 33342 and doxorubicin, visualized at 365 and 546 nm, respectively). Sections were fixed in 50% (v/v) acetone/methanol for 10 min at room temperature and endothelial cells were stained using an antibody to PECAM/CD31 (BD Pharmingen) and fluorescent Alexa 647 secondary antibody (Invitrogen). Reduced EF5 adducts in viable hypoxic cells were stained using the monoclonal antibody specific for EF5 adducts, ELK3-51 (21), followed by a fluorescent Alexa 488 secondary. Evaluation of cell density was carried out by imaging sections following their immersion in Hoechst 33342 (8 μg/mL at 37°C) for 30 min.

Analysis of hypoxia using flow cytometry. The methods used for analyzing the hypoxic fraction of cells present in solid tumors were reported previously (22, 23). Harvested tumors were rinsed in HBSS (Stem Cell Technologies) containing 0.4% bovine serum albumin. A portion of the tumor (~25-30%) was chopped with scalpels in ice-cold HBSS-0.4% bovine serum albumin and transferred to 14 mL tubes with disaggregation mixture [HBSS-1% bovine serum albumin fraction V (Calbiochem) collagenase type 2 and 4, final concentration 250 units/mL (Worthington Biochemical)] and rotated at 37°C for 2 h. The resulting suspensions were placed in 50 μm Medicones and run (3×, 1 min each) through a Medimachine (BD Biosciences) with extensive PBS-0.1% bovine serum albumin (PBSB) washes after each run. Collected cells were washed once with PBSB. Pellets were resuspended in 2.5 mmol/L EDTA and incubated at 37°C for 5 min to reduce cell clumping. Lastly, cells were washed, resuspended in PBSB, and stored on ice for subsequent processing. Tumor cell suspensions were diluted in 0.1% trypan blue in PBS and cells were counted using a hemocytometer. Fragments of cells, erythrocytes, and cells with 2× erythrocyte volume or smaller were excluded. All flow cytometric analyses were done using the FACSCalibur (Becton Dickinson). Hypoxia was measured by detecting EF5 adducts using the ELK3-51-Cy5 antibody developed and generously provided by Dr. C.J. Koch et al. (University of Pennsylvania). Tumor cells were fixed with 2% FA, permeabilized with 1% Tween 20, and blocked overnight to reduce nonspecific binding. ELK3-51-Cy5 antibody was titrated, and the concentration with the best signal-to-noise ratio was chosen for staining. Fixed cells (5×10^6) in 300 μL were incubated with ELK3-51-Cy5 antibody (room temperature for 3 h on a rotator) and washed three times with PBSB-0.5% Tween 20, with the third wash rotating for 1 h. Finally, cells were washed and resuspended in PBSB with 1 μg/mL Sytox Green (DNA dye; Molecular Probes) and analyzed the same day on a flow cytometer. Controls for this assay consisted of cells cultured *in vitro* for 3 h with or without 200 μmol/L EF5 in normoxic (air) or hypoxic (0.005% O_2 + 99.995% N_2) conditions. Mouse cells were differentiated from tumor cells based on DNA content compared.

Image acquisition and analysis. The imaging system consists of a robotic fluorescence microscope (Zeiss Imager Z1), a cooled, monochrome CCD camera (Retiga 4000R, QImaging), a motorized slide loader and x-y stage (Ludl Electronic Products), and customized NIH-ImageJ software.[7] The system allows adjacent microscope fields of view to be photographed and automatically tiled to produce a montage of the entire tumor cryosections at a resolution of 0.75 μm/pixel for qualitative and quantitative analysis. All variables stained on the same section were imaged separately using the monochrome camera and subsequently overlaid and aligned to generate false-color images using Adobe Photoshop (CS) or for quantitative analysis examining the spatial relationships between two and three factors of interest. NIH software applications and user-supplied algorithms were used to quantify the degree of staining on images by measuring the percentage of pixels above a threshold, determined to be a minimum of SDs above background, for the markers CD31 (>7 SDs above background) and EF5 (>7 SDs above background), and intravenously administered perfusion marker Hoechst 33342 (>18 SDs above background). Accumulation of doxorubicin was quantified by determining the average intensity of doxorubicin native fluorescence for pixels located within the tumor margins, and the data were normalized to the average intensity of flooded Hoechst 33342 staining as a control for cell density. Background autofluorescence was determined similarly using tumors untreated with doxorubicin, and this average value was subtracted from doxorubicin-treated tumors. A Leica DLM-100 microscope with a RGB filter was used to image formalin-fixed, H&E-stained sections.

Magnetic resonance imaging and spectroscopy. All magnetic resonance experiments were carried out using a 7.0 Tesla MR scanner (Bruker). Signal transmission and reception was achieved with a three-turn solenoidal radiofrequency coil (1.7 cm inner diameter) with the tumor situated in its interior. This coil was tuned to the hydrogen proton frequency (300.3 MHz) for the K_{trans} measurements and to the ^{19}F frequency (282.58 MHz) for the 5-FU measurements. The K_{trans} values were obtained from serial images acquired to monitor changes in the concentration of a MR-visible contrast agent (Gd-DTPA) within each pixel during the initial uptake and subsequent washout of the agent in the tumor. The magnetic resonance imaging scans follow the protocol reported by Lyng et al. (24); briefly, mice were anesthetized with isofluorane (5% induction, 2% maintenance), a catheter was inserted into the lateral tail vein, and the tumor was placed in the solenoid coil. A proton-density weighted scan was first acquired to

[7] Public domain program developed at the NIH (http://rsb.info.nih.gov/nih-image) running on a G5 Macintosh computer (Apple).

serve as a baseline for conversion of pixel intensity to absolute concentration values of the contrast agent. A volume equivalent to 10 μL/g body weight of the contrast agent (0.3 mmol/kg Gd-DTPA in saline) was injected via the tail vein catheter for 15 to 25 s. Starting at the time of injection, a series of 41 consecutive T_1-weighted scans was acquired with each scan lasting 64 s (spin echo MSME, TR/TE = 11.9/500 ms, field of view = 4 cm, matrix = 128 × 128, slice thickness = 1.5 mm, number of slices = 12). The concentration-time curve for each pixel was fit to a two-compartment Kety model (25), which describes the pharmacokinetics of the contrast agent using two variables: v_e (volume of extracellular extravascular space) and K_{trans} (volume transfer constant between the vasculature and tissue compartment). To assess the relative amounts of 5-FU present within treated and untreated tumors in a second group of mice, 0.05 mL of 0.12 mol/L trifluoroacetic acid in a small glass sphere, placed at the bottom of the solenoid, was used as a fluorine reference. Animals were immobilized with ketamine and acepromazine (175 and 6 mg/kg, respectively). 5-FU (200 mg/kg) was injected intravenously 1 min before the start of spectroscopy measurements. Nonlocalized fluorine spectra were acquired with a bandwidth of 50 kHz, 8192 digitizer points, repetition time of 1 s, and 300 averages, leading to a time resolution of 5 min. The evolving 5-FU peak was observed at 94.1 ppm down-frequency from the trifluoroacetic acid peak. The ratio of integrals between the 5-FU peak and the trifluoroacetic acid peak was calculated to estimate the relative amount of 5-FU present in the tumor at a particular time. Scans were repeated up to a maximum of 2 h. One hour after ^{19}F MRS scans, animals were sacrificed and the tumors were harvested. The tissue was weighed and digested in 500 μL Solvable at 50°C overnight (Sigma) before the addition of EDTA (200 mmol/L, 50 μL), H_2O_2 (30%, 200 μL), and HCl (10 N, 25 μL). The digested tissue was then added to 5 mL scintillation fluid and placed in a scintillation counter (Packard Tri-carb LS 1900 TR) to evaluate the presence of bound [^{14}C]5-FU present per gram tumor tissue.

Expression levels of VEGF-A, VEGF-C, TIMP-1, and interleukin-8. Tumors were homogenized in lysis buffer (150 mmol/L NaCl, 1% NP-40, 0.5% sodium deoxycholate, 2.5 mmol/L EDTA, 0.1% SDS, Mini protease inhibitor cocktail tablets from Roche Diagnostics) using a Polytron homogenizer (Kinematica) and stored at -80°C. Protein determination was done in triplicate using a Micro BCA protein assay (Pierce). Portions of the tumor lysates were pooled and used with the TransSignal Angiogenesis Antibody Array (Panomics) to assay relative changes in proangiogenic and antiangiogenic factors according to the manufacturer's instructions. For Western blots, ~60 μg total protein was loaded and weight separated on a NuPAGE 4% to 15% Bis-Tris gel (Invitrogen). Protein was transferred to 0.45 μm nitrocellulose membranes (Invitrogen) and blocked with 5% skim milk powder in TBST [150 mmol/L NaCl, 50 mmol/L Tris, 0.1% Tween 20 (pH 7.5)] for 2 h. The membranes were then probed for VEGF-A (Santa Cruz Biotechnology), VEGF-C (Zymed), and TIMP-1 (Chemicon) expression using rabbit IgG antibodies diluted in TBST with 5% bovine serum albumin (Sigma-Aldrich) at concentrations of 1:5,000, 1:5,000, and 1:1,000, respectively. Membranes were incubated with primary antibodies overnight at 4°C with gentle shaking. Following incubation, the membrane was washed in TBST (3 × 10 min). The corresponding anti-rabbit horseradish peroxidase-conjugated secondary antibody (Promega) was applied at a 1:5,000 dilution in TBST and 3% skim milk for 1 h. The membrane was washed again in TBST (3 × 10 min) and covered with enhanced chemiluminescent solution (Amersham Biosciences). After 1 min, excess enhanced chemiluminescent solution was poured off, and the membrane was sandwiched in a transparent sheet protector. Bands were then visualized using autoradiography film (Biomax MR Film; Kodak) in a safe-light darkroom. Tumor lysates were analyzed for levels of interleukin-8 (IL-8) using a human CXCL8/IL-8 ELISA (Quantikine; R&D Systems). All samples consisted of 160 μg total protein and were done in duplicate according to the manufacturer's protocol. The standard curve was generated using a four-variable logistic curve fit.

Statistics. Statistical analyses were done with Statistica software. One-way ANOVA was used to calculate P values. Differences were considered significant at $P \leq 0.05$.

Results

Irinophore C treatment inhibits growth and reduces the cell density of HT-29 tumors. All data presented were derived from tumors harvested from mice treated for 6 weeks. The inhibitory effect of Irinophore C on subcutaneous HT-29 tumors is shown in Fig. 1A. As outlined in Materials and Methods, mice were randomly assigned to 7 different groups when tumors were ~150 to 200 mm^3. Mice in the control group were treated with saline (□), and mice in the remaining groups were treated with Irinophore C for 3 weeks (●) or for 6 weeks with a 12-day break after week 3 (■). Treatment with Irinophore C inhibited tumor growth but did not cause tumor regression at this dose. When treatment was suspended, the tumors started to grow again within 5 to 8 days (●). If treatment was started again (■), further stabilization of the tumor was observed. Mice in the 6-week treatment group were sacrificed following imaging procedures to determine K_{trans}, and the tumors were collected for analysis as specified in Materials and Methods.

Tumor cell density was assessed by staining cell nuclei with Hoechst 33342. Image analysis of these sections indicated that the average intensity of Hoechst 33342 staining, summarized in Fig. 1B, was significantly lower for those tumors treated with 6 weeks of Irinophore C (■) (~35% lower; $P = 8.42 \times 10^{-5}$) compared with untreated tumors (□), reflecting a lower density of nuclei or cell density. H&E-stained tumor sections obtained from animals treated with saline or Irinophore C are shown in Fig. 1C. The tissue structure in untreated tumors is composed of densely packed cancer cells whose nuclei are stained dark purple and are permeated with river-like bands of stroma (pink). However, after treatment with Irinophore C, the density of the cancer cells is much lower and the tumor cells appear to be grouped in small islands amidst what appears to be fatty tissue, as determined by an experienced pathologist. Tumor cell nuclei from treated tumors are larger and lack prominent nucleoli, which are easily detected in tumors from saline-treated animals. Necrosis was not widespread or confluent and confined to single cells.

Irinophore C treatment is associated with a decrease in K_{trans}. Noninvasive magnetic resonance imaging was used to assess K_{trans}, the volume transfer constant of a solute between the blood vessels and extracellular tissue compartment of the tumor. The median viable values of K_{trans} for the tumors within the control and treated groups are graphed individually in Fig. 2A and show the relative spread of K_{trans} values between the control group (□) and the Irinophore C-treated group (■). The average values for K_{trans} in untreated tumors was ~1.5 times greater than in treated tumors (0.0375 and 0.025 mL/g/min for untreated and treated tumors, respectively; $P = 0.050$). The values for K_{trans} in untreated tumors were more variable compared with the treated group (SD = ±0.01 and ±0.001, respectively).

Irinophore C treatment is associated with increases in Hoechst dye perfusion and in the CD31$^+$ cells to tumor cell ratio. Hoechst 33342 dye was injected intravenously into the lateral tail vein

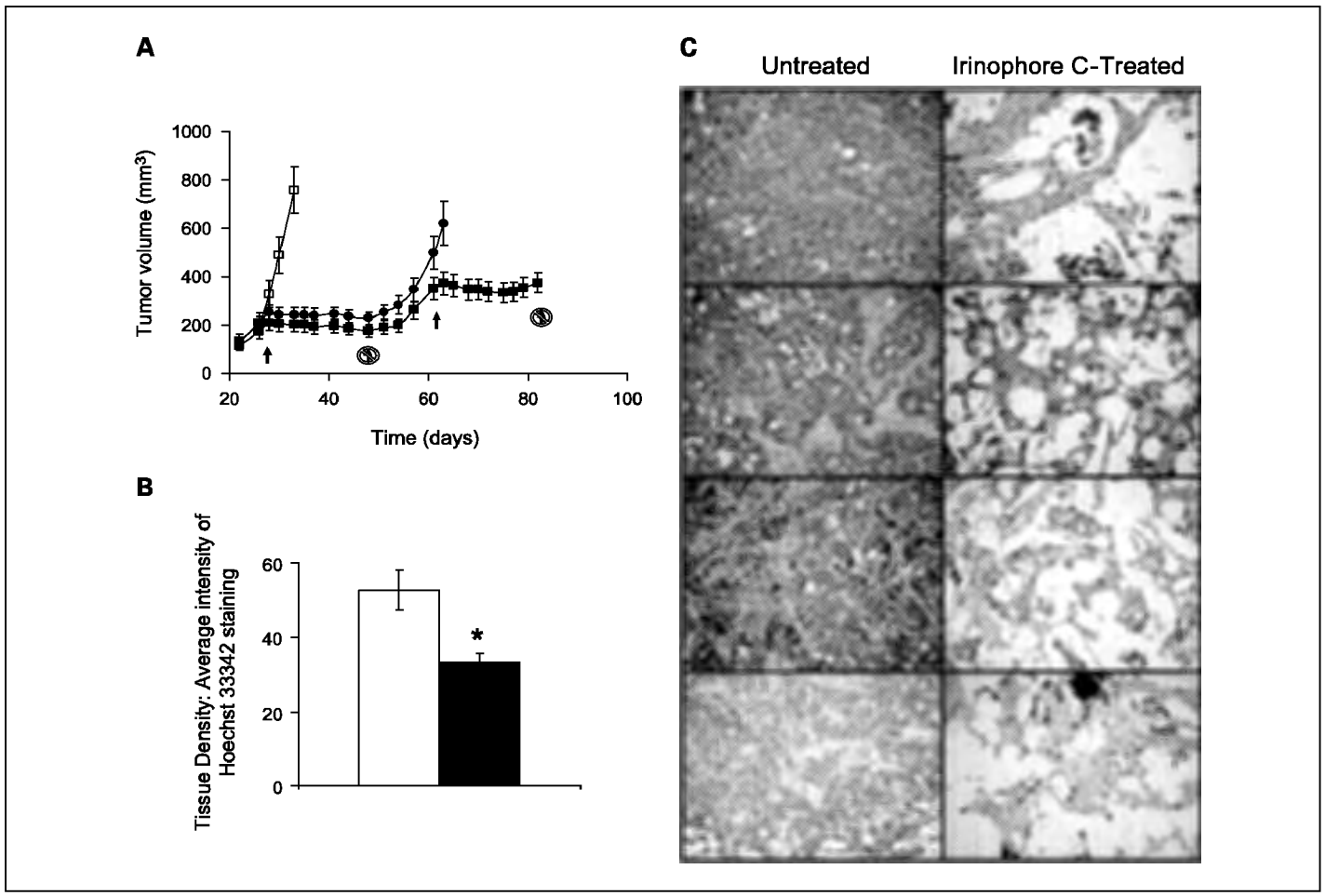

Fig. 1. *A* to *C*, Irinophore C treatment has a cytostatic effect on HT-29 tumors and reduces the cell density of the tumor. *A*, inhibitory effect of Irinophore C on HT-29 tumors grown subcutaneously in Rag2M mice. *Arrows*, start and end of treatment. Tumors in mice treated with saline reached predetermined endpoints within 2 wk and were harvested immediately (□). Tumor growth in mice treated with Irinophore C for 3 wk (●) or 6 wk with a 10- to 12-day break after week 3 (■) was inhibited, but the tumor was not eradicated. When treatment was suspended, the tumors started growing again (●); however, when treatment recommenced, growth was inhibited again (■). *B*, cell density of tumors treated with 6 wk of Irinophore C (■) was significantly reduced compared with untreated tumors (□). Cryosections of the tumors were flooded with Hoechst 33342 to stain cell nuclei as a measure of cell density. The average intensity of Hoechst 33342 staining shows that the cell density in treated tumors is reduced by ~35% ($P = 8.42 \times 10^{-5}$). *C*, images of H&E-stained tumor sections (magnification, ×20) from 4 sets of control and treated tumors show representative fields of view, clearly illustrating that the effect of Irinophore C is consistent in dramatically changing the tissue morphology and reducing the cell density.

of animals 20 min before tumor harvest to assess perfusion within the tumor. Significantly higher amounts of the fluorescent DNA-binding dye were found in tumors treated with Irinophore C. Figure 2B shows representative false-color composite images of cryosections from HT-29 tumors; Irinophore C-treated tumors contain greater quantities of dye labeling (blue) compared with controls. The proportion of pixels per unit area with staining intensity greater than threshold for Hoechst 33342 were quantified, and the data show that there is ~2.8-fold more Hoechst 33342 labeling in tumors from Irinophore C-treated animals (Fig. 2C; $P = 0.022$). The cryosections from HT-29 tumors shown in Fig. 2B were also stained for $CD31^+$ cells (green), and the results indicate that treatment with Irinophore C significantly decreased the number of $CD31^+$ pixels present in tumor sections. More specifically, tumor mapping analysis of entire sections showed that the overall percentage of $CD31^+$ pixels above threshold, as summarized in Fig. 2D, was significantly reduced ($P = 0.014$) in tumors from Irinophore C-treated animals (■) compared with untreated controls (□). Furthermore, when the percentage of $CD31^+$ pixels was normalized to the cell density, the results show a 1.8-fold increase in $CD31^+$ pixels relative to the number of tumor cells in the treated tumors compared with controls ($P = 0.00024$; Fig. 2E).

Irinophore C reduces tumor hypoxia. The level of tissue oxygenation in HT-29 tumors from control and Irinophore C-treated animals was examined with the hypoxic marker EF5. Because control, untreated tumors were substantially larger than treated tumors, the levels of hypoxia in the HT-29 model were first examined as a function of size. Untreated HT-29 tumors ranging from 200 to 600 mm^3 in size were exposed to EF5 as described in Materials and Methods. Subsequently, these tumors were disaggregated and analyzed with flow cytometry for the presence of viable hypoxic cells as reported previously (23). The results, plotted as a function of volume, are summarized in Fig. 3A and indicate that the percentage of viable hypoxia tumor cells is not correlated to size in HT-29 tumors ($R^2 = 0.0766$) within the range of volumes examined.

These results seemingly contradict previously published data where HT-29 tumors <1 mm in diameter were shown to be intensely hypoxic (and avascular) compared with tumors 1 to 4 mm in diameter (26); however, the size of tumors used in these studies were considerably larger (>0.5 cm^3). As shown in Fig. 3B, untreated tumors analyzed with flow cytometry were found on average to have a population of viable hypoxic levels of ~18% (consistent with the results shown in Fig. 3A), whereas treatment with Irinophore C for 6 weeks significantly reduced the population of viable hypoxic cells by 3-fold (~5% viable hypoxic cells; $P = 5.32 \times 10^{-9}$). Representative HT-29 sections for tumors from untreated and treated animals are shown in Fig. 3C and D, where a false-color image depicting cells stained positive for EF5 (red) is overlaid with an image for $CD31^+$ endothelial cells (green) against a hematoxylin background (gray). The images show that tumors from saline-treated animals have larger areas of hypoxic cells compared with tumors obtained from Irinophore C-treated animals.

Irinophore C treatment increases the accumulation of a second drug. Increased accumulation of Hoechst 33342 (Fig. 2C) and improvements in oxygenation levels (Fig. 3B-D) led us to investigate the effects of Irinophore C treatment on the delivery of 5-FU. The appearance of 5-FU in HT-29 tumors from animals treated with Irinophore C or saline was monitored with ^{19}F spectroscopy noninvasively over time in live animals. The doses used in this study were not meant to reflect therapeutically relevant doses but chosen to ensure a good signal-to-noise ratio for ^{19}F MRS. The amplitudes of the peak corresponding to the single fluorine atom in 5-FU, relative to an external standard (trifluoroacetic acid) and normalized to the tumor size, in individual control (□) and treated (■) mice are shown in Fig. 4A. The data suggest that the appearance of 5-FU is more variable in Irinophore C-treated tumors but that the tumors are exposed to up to 10 times more drug compared with untreated controls over the same period. The levels of bound ^{14}C-labeled 5-FU, added at tracer levels to the injected drug, in tumors from saline-treated (□) or Irinophore C-treated (■) animals were also measured using scintigraphy. The results show that ~1.5 times more 5-FU was present per gram of tumor tissue in tumors from Irinophore C-treated animals compared with

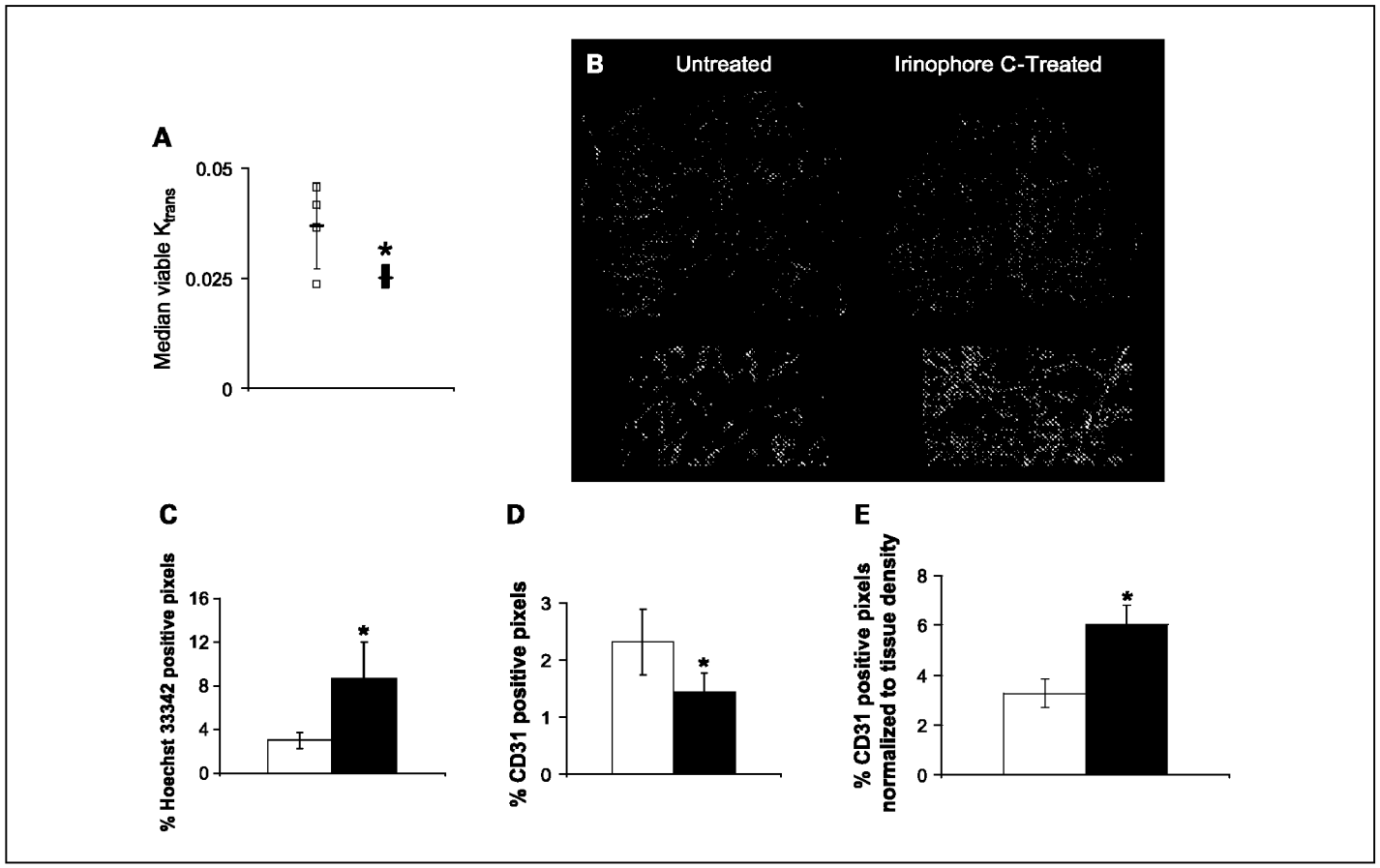

Fig. 2. *A* to *E*, vascular function in HT-29 tumors is improved with Irinophore C treatment. *A*, median viable values for K_{trans} in individual HT-29 tumors show the relative spread of K_{trans} values between the control group (□; 4 animals) and Irinophore C-treated group (■; 5 animals). The average value for K_{trans} (-) was significantly lower in the treated group ($P = 0.050$). The values for K_{trans} were more variable for the untreated group compared with the treated group of mice (SD = ±0.01 and ±0.001, respectively). *B*, significantly higher amounts of the fluorescent DNA-binding dye Hoechst 33342 injected intravenously are delivered to HT-29 tumors following treatment with Irinophore C relative to untreated controls. Representative images of sections from tumors harvested 20 min after intravenous injection of Hoechst 33342 (*blue*) show that more dye is present and that the overall amount of CD31 staining (*green*) is decreased in treated tumors. *C*, percentage of Hoechst 33342-positive pixels in sections from control (□) and treated tumors (■) was quantified, and the data show that ~2.8-fold more Hoechst 33342 is present in the Irinophore C-treated tumors ($P = 0.022$). *D*, tumor mapping analysis of sections stained specifically for endothelial cells indicates that the percentage of $CD31^+$ pixels per section in Irinophore C-treated tumors (■) is significantly reduced ($P = 0.014$) compared with untreated controls (□). Additional CD31 images can be seen in Figs. 3C and 4C. *E*, when the percentage of $CD31^+$ pixels is normalized to cell density (Hoechst 33342 flooding), a net 1.8-fold increase is seen ($P = 0.00024$) following treatment with Irinophore C.

controls (Fig. 4B; $P = 0.0002$). The scintigraphy data confirm, at a single time point, the ^{19}F spectroscopy data.

The accumulation of another commonly used anticancer drug, doxorubicin, was also evaluated in HT-29 tumor-bearing animals that were treated with saline or Irinophore C. Animals were injected with a single dose of doxorubicin (30 mg/kg) in animals treated with saline or with 6 weeks of Irinophore C. Size-matched tumors were subsequently harvested and the native fluorescence of bound doxorubicin present in the sections was visualized with fluorescence microscopy before subsequent staining and reimaging for endothelial cells (CD31) and nuclear density (Hoechst 33342 flooding). Representative false-color images of tumor sections from mice injected with doxorubicin are shown in Fig. 4C, where the native fluorescence of doxorubicin in tumors from mice treated with Irinophore C had a greater distribution compared with untreated control tumors. It should be noted that the cell density is not discernible in these images, and gaps in doxorubicin fluorescence within the tumors from Irinophore C-treated animals were associated with low tumor cell density (see Fig. 1B). Consequently, the visual assessment was corroborated by normalizing the average intensity of doxoru-

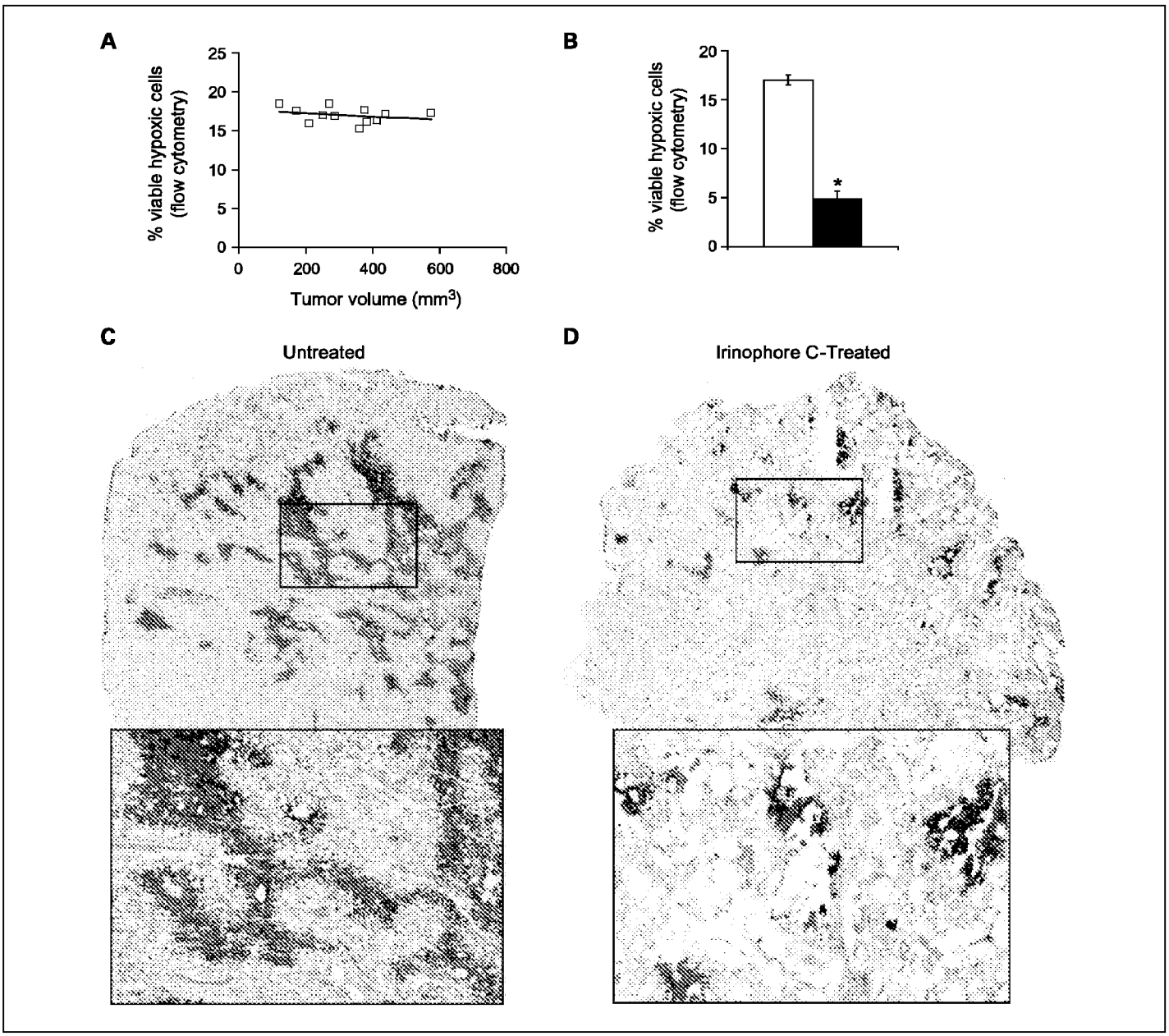

Fig. 3. *A* to *D*, Irinophore C treatment reduces levels of hypoxia in HT-29 tumors. *A*, percentage of viable hypoxic cells in untreated tumors ranging in size from 200 to 600 mm^3 was determined with EF5 staining and flow cytometry. No correlation was found between levels of hypoxia and the size of the tumor ($R^2 = 0.0766$). *B*, ~18% of viable cells in untreated tumors were found to be hypoxic; in contrast, Irinophore C-treated tumors were better oxygenated containing a significantly smaller population of viable hypoxic cells (~5%; $P = 5.32 \times 10^{-9}$). *C* and *D*, images of representative HT-29 sections with hypoxic and endothelial cells colored red and green, respectively, against a hematoxylin background show that untreated tumors have larger areas of hypoxic cells compared with the treated tumors. *Squares*, magnified images immediately below each section.

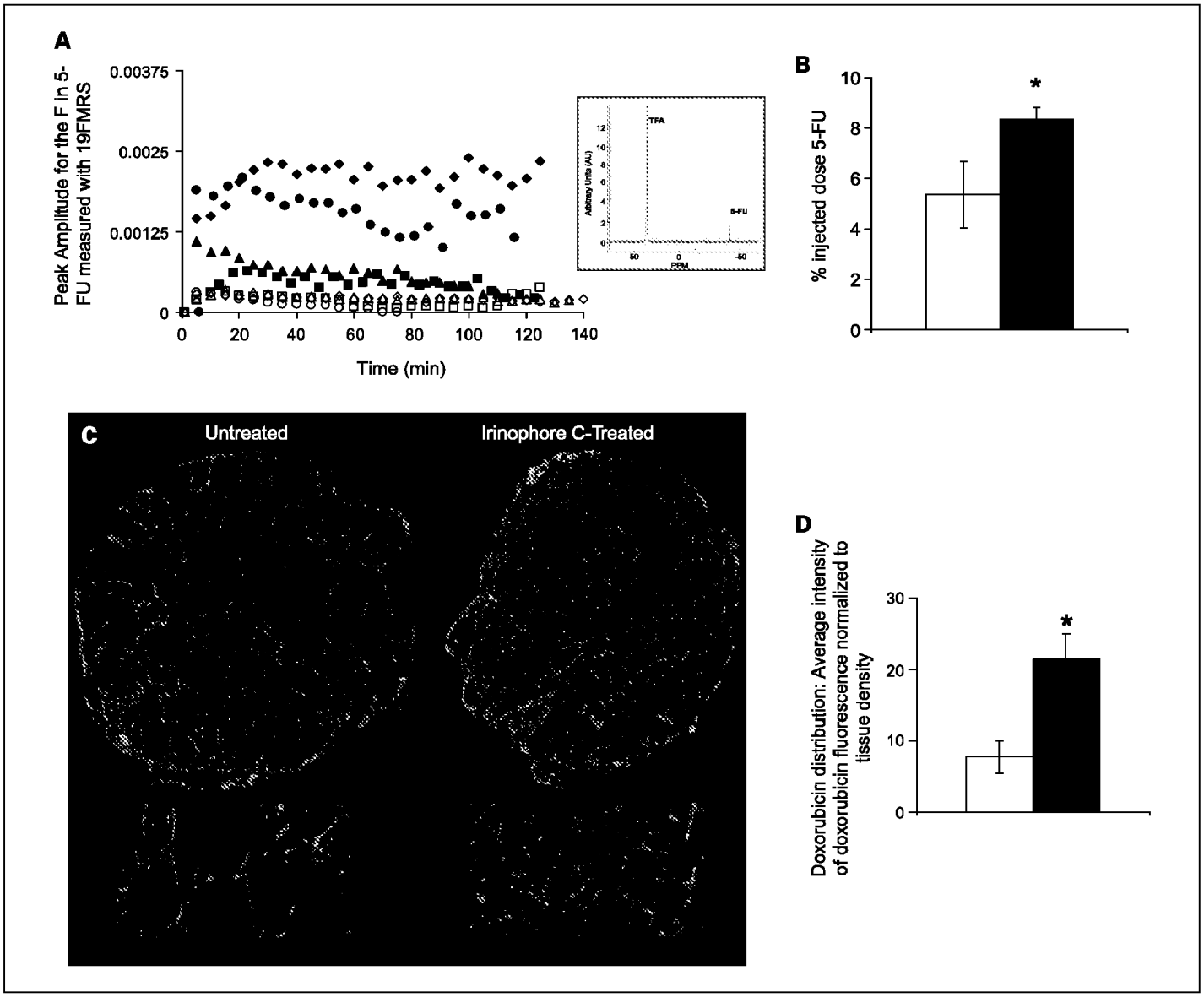

Fig. 4. *A* to *D*, treatment with Irinophore C increases the accumulation of a second drug in HT-29 tumors. *A*, ^{19}F spectroscopy was used to evaluate the delivery of 5-FU noninvasively in HT-29 tumors over 2 h in living animals. The relative amplitudes of the peak corresponding to the single fluorine atom in the 5-FU, normalized to an external standard (trifluoroacetic acid), in individual animals from untreated groups (□) and treated groups (■). *Inset*, representative spectrum for 5-FU measured *in vivo*. Data indicate that exposure of the tumor to 5-FU is more variable in Irinophore C-treated tumors but that higher concentrations of drug are present compared with untreated controls (up to 10-fold more drug in the same period). *B*, levels of [^{14}C]5-FU, added at tracer levels to the injected drug dose for ^{19}F MRS, bound to untreated tumors (□) and Irinophore C-treated tumors (■) were measured using scintigraphy. The percent injected dose of 5-FU present in the tumor tissue was ~1.5 times greater in Irinophore C-treated tumors compared with controls ($P = 0.0002$). *C*, similar results were obtained with the anticancer drug doxorubicin; control and Irinophore C-treated animals were injected with a single dose of doxorubicin, and the tumors were subsequently harvested and analyzed with fluorescence microscopy. The images of the tumor sections show that more doxorubicin is present in the Irinophore C-treated tumor tissue compared with untreated controls. *D*, average intensity of doxorubicin-positive pixels normalized to the cell density (Hoechst 33342 flooding) shows that Irinophore C-treated tumors (■) have significantly more doxorubicin present (~2.7-fold increase; $P = 0.2.43 \times 10^{-5}$) compared with control tumors (□).

bicin native fluorescence to the cell density as measured by the average intensity of flooded Hoechst 33342 staining. As summarized in Fig. 4D, in tumors from Irinophore C-treated animals (■), the amount of doxorubicin is ~2.7-fold greater than in control tumors (□; $P = 0.\ 2.43 \times 10^{-5}$).

Antiangiogenic and antivascular effects of Irinophore C. To better understand the observed effects of Irinophore C on tumor vasculature (see Fig. 2B and D), a survey of promoters and inhibitors of angiogenesis was completed. The results obtained from an ELISA screen of proangiogenic and antiangiogenic activators are summarized in Table 1. Two proangiogenic factors, VEGF and IL-8, were down-regulated and an inhibitor of angiogenesis, TIMP-1, was up-regulated in tumors from Irinophore C-treated animals compared with saline-treated controls. Other markers in the array were not detected by this assay, so no conclusions can be drawn regarding these activators and inhibitors. To confirm results obtained in the ELISA screen, Western blot analysis for VEGF

and TIMP-1 in tumor lysates from individual animals treated with saline or Irinophore C was completed. The results, shown in Fig. 5, corroborate the results of the initial screen for two forms of VEGF and for TIMP-1. An ELISA kit for human CXCL8/IL-8 (Quantikine; R&D Systems) was used to assess the levels of IL-8 in the treated and control tumors (154 and 265 pg/mL, respectively); a strong trend toward lower levels of IL-8 in treated tumors (~1.7-fold less) was observed, although the results did not achieve significance ($P = 0.07$).

Discussion

Lipid-based nanopharmaceuticals are reasonably well-established (27–29), and in the context of anticancer drugs, most investigators would suggest that the benefits of these drug carriers include prolonged systemic drug exposure, enhanced delivery of the associated drug to tumors, and/or protection of the associated drug from premature metabolism in the plasma. Drug carrier formulations of camptothecins have been aggressively pursued in part because the formulations improve the availability of the active lactone form of the drug (30). The liposomal formulation of irinotecan developed by our group (Irinophore C) maintains the drug in its active form within the liposome, extends its plasma half-life, and improves accumulation of drug in the tumor (16). Prolonged systemic exposure to the active form of irinotecan as well as its more active metabolite SN-38, along with other data suggesting that these drugs can have antivascular or antiangiogenic effects, prompted us to study the effects of Irinophore C on tumor morphology and vasculature. We recently reported that Irinophore C was significantly more active than irinotecan in 5 different xenograft models (17), so here we focus on one of those tumor models (HT-29) and completed a multimodality imaging analysis of tumor-associated vascular structure and function. The results of this study clearly show that Irinophore C treatment has a striking effect on the morphology and vasculature of the tumor. The changes in vascular structure and function have important implications for using this drug in a combination setting and, as discussed below, allow us to speculate on the potential use of this nanopharmaceutical to increase the penetration and accumulation of a second chemotherapeutic agent.

Table 1. Irinophore C treatment of HT-29 tumors inhibits angiogenesis

Activators			Inhibitors
ANG	IL-1α	FGF-α	IFN-γ
G-CSF	IL-1β	FGF-β	IL-12
HGF	IL6	TNF-α	IP-10
Leptin	*IL-8*	TGF-β	**TIMP-1**
VEGF	PIGF	*Negative controls*	TIMP-2
		Positive controls	

NOTE: Pooled tumor lysates from untreated and treated tumors were assayed with an ELISA-based screen of proangiogenic and antiangiogenic activators. The proangiogenic factors VEGF and IL8 were down-regulated in Irinophore C-treated tumors relative to untreated controls (italicized), whereas the inhibitor of angiogenesis TIMP-1 was up-regulated (bold). The remaining factors in the assay were not detected in either group of tumors.

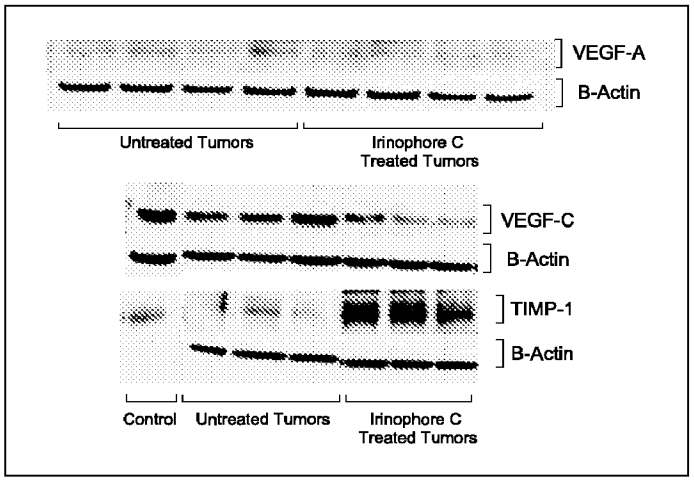

Fig. 5. Irinophore C treatment of HT-29 reduced expression levels of VEGF A and C but up-regulated expression levels of TIMP-1. Western blot analysis of the treated tumor lysates confirm that VEGF-A and VEGF-C were down-regulated (~2- and ~3.5-fold, respectively), whereas TIMP-1 was up-regulated (~4-fold) compared with untreated tumors.

It is notable that Irinophore C treatment stabilized the disease and did not cause tumor regression at these doses (Fig. 1A). This is similar to data that we have generated with a lipid-based formulation of vincristine, which when used to treat a human squamous cell carcinoma resulted in stable disease over an extended time course (31). Treatment effects that are associated with the appearance of stable disease are consistent with the observed effects obtained with metronomic chemotherapy (32, 33), where low doses of anticancer drugs are administered on a frequent or continuous schedule without extended breaks (33). The mechanistic basis of metronomic chemotherapy is believed to be primarily antiangiogenic, from either direct kill of endothelial cells in the growing tumor vasculature (34, 35) and/or destruction of bone marrow-derived endothelial progenitor cells (36, 37). The effects on tumor-associated endothelial cells is not necessarily surprising because many anticancer drugs, including irinotecan, have preferential activity against endothelial cells *in vitro* (38). In fact, the free drug was examined as an antiangiogenic agent in the late 1990s (39, 40) and was shown to inhibit neovascularization in a cornea model of angiogenesis (40). More recently, irinotecan was used to examine angiogenesis in a green fluorescent protein transgenic nude mouse model of human colon cancer (18). The use of irinotecan in a metronomic dosing regimen in a preclinical model of colorectal cancer (HT-29) was recently described (41); the authors indicate that metronomic dosing with irinotecan alone significantly inhibited tumor growth and also decreased microvessel density. The same group has now examined the pharmacokinetic and pharmacodynamic effects of metronomic irinotecan in metastatic colorectal patients, although the study did not include an examination of antivascular effects in the tumor tissue (42). Irinophore C, which behaves as a circulating drug depot that maintains low doses of active drug in the circulation for extended periods, may in fact be fundamentally akin to metronomic chemotherapy. We therefore speculate that the prolonged exposure to irinotecan and its active metabolite SN-38 (17) following intravenous use of Irinophore C may

elicit effects consistent with those achieved with metronomic dosing (42). Liposomal formulations of doxorubicin have also been shown to produce antivascular activity in models of glioma (43), which further corroborates our postulate that the liposomal formulation of Irinophore C indeed does have antiangiogenic effects.

Treatments with inhibitory effects on tumor vasculature have also been postulated to transiently and partially "normalize" the typically torturous, redundant, and inefficient vasculature found in tumors (44, 45). The concept of "vascular normalization" was originally conceived in 1972 (46) and more recently described in terms of a rebalancing of angiogenic processes gone awry in tumor growth (45). Whatever the precise mechanism behind this process, normalization of vasculature in window dorsal chamber tumor models treated with agents against VEGRF-2 improves blood flow and oxygen delivery to the cancer cells. These changes are associated with subsequent increases in drug penetration (47) and the sensitivity of the cells to radiation (48). Efficient delivery and distribution of anticancer agents is crucial in the treatment of solid tumors where every cancer cell, regardless of its local microenvironment, must be exposed to toxic levels of drug (49). Many small-molecule cancer therapeutics have shown evidence of poor tumor tissue penetration in both *in vitro* and *in vivo* model systems, including doxorubicin (50), gemcitabine (51), docetaxel, and paclitaxel (52). Thus, the concept of modifying the tumor vasculature, even temporarily, to improve radiotherapy by decreasing hypoxia, or increasing delivery of systemically administered chemotherapeutics, is highly compelling from the clinical perspective. The present studies indicate that Irinophore C treatment significantly decreases the number of endothelial cells and reduces K_{trans} while increasing the accumulation of the perfusion marker Hoechst 33342 in HT-29 tumors. The decrease in CD31 staining in treated tumors is consistent with an antiangiogenic effect (Fig. 2D). However, when CD31 staining is expressed relative to tumor cell density (see Fig. 2E), the ratio of endothelial cells to the number of cancer cells present in the section is actually ~1.8-fold higher in treated tumors. Thus, relative to the size of the tumor, the overall vasculature is decreased; however, in terms of tumor cell density, the ratio of vasculature to tumor cells is increased presumably improving the coverage of the vascular network.

These data suggest that improved delivery of a second compound, as seen here with Hoechst 33342, 5-FU, and doxorubicin, would be expected based on the drug-induced changes in vascular structure and function. We also believe that decreases in K_{trans} values in tumors following treatment with Irinophore C are consistent with improved vascular function. If the vasculature is normalized in treated tumors, and vessels become less chaotic and leaky, then vessel permeability becomes the rate-limiting step for determining K_{trans}, and the values would be expected to drop, as was the case. Thus, the large variability in K_{trans} values associated with untreated tumors (see Fig. 2A) likely reflects the random nature of chaotic and leaky blood vessels in tumors (45). Leaky tumor vasculature allows the MR-visible contrast agent to enter the extracellular matrix easily, so blood flow is the rate-limiting step in the process and K_{trans} values in untreated tumors are more likely to approximate blood flow rates. The reduction of overall vasculature in the treated tumors may seem to be at odds with the levels of Hoechst 33342 delivered and one might expect that fewer blood vessels would be associated with reduced dye delivery to the tumor. However, the possibility that Irinophore C treatment may normalize tumor vasculature, rendering the vessels more functional and thus able to deliver more of the dye, is a reasonable interpretation of the results. Improved vascular function, as evidenced by the K_{trans} data, in combination with the dramatic changes to tissue morphology and density, may explain significantly enhanced accumulation of secondary agents in tumors from animals previously treated with Irinophore C.

The antiangiogenic effect of Irinophore C is corroborated by data showing that two promoters of angiogenesis, VEGF (53) and IL-8 (54), are down-regulated, whereas an inhibitor of angiogenesis, TIMP-1 (55), is activated in the treated tumors (see Table 1). VEGF-A is known to promote endothelial cell proliferation, sprouting, and tube formation; VEGF-C also contributes to angiogenesis by activating VEGF receptors (53). VEGF levels have also been correlated with vessel permeability (56); lower VEGF levels in treated tumors would be expected to decrease blood vessel permeability, further supporting the interpretation of the observed K_{trans} values. Evidence also exists for IL-8 being a stimulus for endothelial cell proliferation, tube formation, and endothelial cell survival (54). In contrast, TIMP-1 is a known inhibitor of matrix metalloproteinases, which are necessary for breaking down the extracellular matrix to permit endothelial cell invasion (55); an increase in expression levels of TIMP-1 would thus have an antiangiogenic effect by preventing this process. However, angiogenesis is a complex process and the precise molecular mechanisms behind the effects of Irinophore C on tumor vascular function is a focus of ongoing studies.

Because treatment with Irinophore C appeared to improve vascular function and accumulation of Hoechst 33342, the effect of treatment on hypoxia as well as the delivery and accumulation of a second drug in these tumors was examined. Tumor hypoxia in tumors from saline-treated and Irinophore C-treated tumors was evaluated with the EF5/ELK3-51 system, which is specific for viable hypoxic cells (20, 21). The data indicate that Irinophore C treatment significantly reduces the proportion of viable, hypoxic tumor cells and this may be equated with improved delivery of oxygen. A decrease in hypoxia would have important implications for scheduling radiation treatments in colon cancer as hypoxia adversely affects radiation treatment (57, 58). Enhanced accumulation and improved distribution of doxorubicin and 5-FU in HT-29 tumors from animals treated for 6 weeks with Irinophore C were significantly higher compared with untreated controls. Exposure of cancer cells to higher levels of either drug would presumably result in better cell kill, and it will be important to now study in preclinical models the influence of Irinophore C treatment in combination with second agents, either given simultaneously or sequentially. More specifically, the results reported here raise the intriguing possibility of using subsequent or concurrent therapies to take advantage of changes in the tumor microenvironment engendered by Irinophore C. Because 5-FU and irinotecan are used in combination (FOLFIRI) to treat colon cancer (3), restoring or improving vascular function in the tumor by replacing irinotecan with Irinophore C could be beneficial if it

improved the therapeutic efficacy of 5-FU by increasing the delivery and subsequent accumulation of the drug in the tumor. Likewise, the efficacy of ionizing radiation could be increased if tumor hypoxia is decreased by treatment with Irinophore C in an adjuvant setting. Our group thus believes that the potential for Irinophore C to change the tumor microenvironment and render cancer cells more vulnerable to sequential chemotherapy or radiotherapy is a novel observation with immediate implications for the treatment of advanced colon cancer. Although the results reported here are relevant to colon cancer, the basic principles should be applicable to all solid tumors because angiogenesis and the expansion of a vascular network is a requirement for tumor proliferation and metastasis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Irinophore C, a Novel Nanoformulation of Irinotecan, Alters Tumor Vascular Function and Enhances the Distribution of 5-Fluorouracil and Doxorubicin

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Oncology

Phase II study of first-line FOLFIRI for progressive metastatic well-differentiated pancreatic endocrine carcinoma

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ABSTRACT

Background: Pancreatic endocrine carcinomas are rare and heterogeneous. Published results concerning treatment of advanced tumours are inconsistent and responses to standard chemotherapy remain unsatisfactory.
Aim: To investigate the ability of the FOLFIRI regimen to manage progressive unresectable metastatic well-differentiated endocrine carcinomas of the pancreas as first-line chemotherapy.
Methods: 20 patients with metastatic or advanced well-differentiated endocrine carcinomas of the pancreas and progressive disease were enrolled in a prospective multicentre phase II trial to receive chemotherapy with FOLFIRI schedule (irinotecan 180 mg/m^2 infusion combined with simplified LV5FU2) every 14 days. The primary end point was the non-progression rate at 6 months.
Results: The 6-month non-progression rate was 80% (95% confidence interval [56–94%]), with stabilisation in 15 patients and 1 objective response. Overall survival at 24 months was 65% [40–82%]. Median progression-free survival was 9.1 months [6.5–17.3 months]. The median number of administered cycles was 12 [range 1–28]. Grade 3/4 haematologic toxicity occurred in 5 patients (25%) and grade 3 digestive toxicity in 11.
Conclusion: The FOLFIRI regimen, as first-line chemotherapy, achieved stabilisation in most patients whose tumours had been progressing and was well-tolerated. It could be an alternative therapy for advanced well-differentiated endocrine carcinomas of the pancreas.

1. Introduction

Endocrine tumours are rare. Their incidence is 5.3/100,000 inhabitants [1]. These tumours constitute a heterogeneous group in terms of histological characteristics, clinical expression, evolution and prognosis. Histological differentiation, grading and disease stage at diagnosis are the main prognostic factors for survival [1–3].

The treatment of well-differentiated endocrine carcinomas depends on the primary site and tumour burden. Radical surgery is the only curative approach and should be considered for patients with potentially resectable disease, even with metastases [4–7]. For unresectable and progressing well-differentiated endocrine carcinomas of the pancreas, anti-cancer treatments, such as chemotherapy, chemoembolisation or biotherapy are recommended. The classical first-line treatment is based on chemotherapy combining doxorubicin and streptozotocin because of the high response rate (69%) obtained by Moertel et al. [8]. However, their results were not confirmed by later studies [9–11]. Numerous chemotherapies and other treatments (biotherapies, targeted biotherapies, radiotherapy and targeted radionucleide radiotherapy) can been given in this setting and have been included in national and international guidelines (www.tncd.org, www.neuroendocrine.net/guidelines_tnm_classifications.html).
The rarity of these tumours make recruitment of homogeneous and sufficiently large cohorts of patients, to obtain adequate statistical power in clinical trials, difficult.

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The FOLFIRI regimen (a combination of irinotecan, 5 fluorouracil and leucovorin) was evaluated by Ducreux et al. in a phase II study on 20 patients with pretreated metastatic well-differentiated endocrine carcinomas (including 10 with primary pancreatic tumours). Tumour control was observed in 16 patients with an objective response in 1 patient [12]. Their results were very encouraging because most of the patients had been heavily pretreated and tolerance was good.

The aim of this prospective, multicentre, open, phase II study was to assess the efficacy and toxicity of the FOLFIRI regimen as first line chemotherapy for patients with unresectable and progressing well-differentiated endocrine carcinomas of the duodenopancreatic area.

2. Patients and methods

The protocol was approved by the Regional Ethics Committee of Champagne-Ardenne on 24 June 2003. The study was registered at clinical trial.gov. with reference NCT00416767. Written informed consent was obtained for all patients.

2.1. Patients

Patients with a histologically confirmed unresectable well-differentiated endocrine carcinoma of the pancreas (functional or not) were eligible. Other inclusion criteria were: age between 18 and 80 years, WHO performance status (PS) ≤ 2, measurable locally advanced (>50 mm for primary tumour and/or lymph-node metastases) or metastatic disease (>15 mm for hepatic or extrahepatic metastases), progressive disease (>20% increase of measurable lesions or appearance of new lesions according to RECIST V1.0 criteria) [13] within the 6 months preceding inclusion. Metastases had to be histologically proven or positive on somatostatin-receptor scintigrams.

Histological diagnosis of well-differentiated endocrine carcinoma was based on the 2000 WHO criteria [14]. The Ki-67 index had to be $\leq$15% and the mitotic count <10 for 10 high-power fields. These cut-off have been determined before the ENETS grading classification has been published [15]. ENETS TNM classification was also retrospectively applied [15].

Biochemical and haematological laboratory tests had to be adequate to receive chemotherapy: neutrophil count $\geq 1500/mm^3$, platelets $\geq 100{,}000/mm^3$, creatinine level $\leq 135\ \mu mol/l$ and total bilirubin $\leq 30\ \mu mol/l$.

Patients had to be naive from: chemotherapy, radiotherapy (external or internal) and/or chemoembolisation. External radiotherapy was an exclusion criteria only if it concerned a target. Interferon had to be stopped 3 months before inclusion but somatostatin analogues were allowed for functional tumours.

Non-inclusion criteria were: poorly differentiated endocrine carcinomas, Gilbert's syndrome, pregnancy and breast feeding.

2.2. Clinical and biological work-up

Four weeks before enrolment, pretreatment evaluation included full medical history and physical examination (weight, body surface area, WHO PS), standard haematological and biochemical analyses and dosages of chromogranin A and biomarkers, depending on the clinical history and presentation (gastrin, insulin, C-peptide, glucagon, vasoactive intestinal peptide, somatostatin, thyrocalcitonin, serotonin) and complete morphological evaluation that included chest and abdominal computed-tomography (CT) scans or magnetic resonance imaging (MRI), and somatostatin-receptor

2.3. Treatment plan

All patients received FOLFIRI chemotherapy, consisting of irinotecan 180 mg/m^2 infusion on day 1 combined with simplified LV5FU2: a single 2-h infusion of leucovorin 200 mg/m^2 on day 1, followed by a 400-mg/m^2 bolus of 5 fluorouracil, then continuous infusion of 5 fluorouracil 2400 mg/m^2 over 46 h. Cycles were scheduled to be repeated every 14 days using a chemotherapy free-interval scheme.

Forty-eight hours before each chemotherapy cycle, haematological and biochemical analyses, physical examination, including body surface area (weight), and WHO PS were done. All toxicities were also assessed using the National Cancer Institute–common toxicity criteria NCI–CTC version 2.0 (available, http://ctep.cancer.gov). Severe adverse events were also recorded within 24 h of their onset.

Treatment was to be stopped when grade 3/4 toxicity persisted after dose reduction or after 3 weeks without treatment because of toxicity, the tumour progressed under chemotherapy, or withdrawal of consent.

When the tumour stabilised, FOLFIRI was prolonged for another 3 months, then a treatment break could be allowed when stabilisation was confirmed and it lasted until progression. During the treatment break, the tumour response was evaluated every 3 months. If the tumour progressed during the chemotherapy free-interval (treatment break), FOLFIRI could be reintroduced and repeated until any toxicity appeared or progression. For a partial response, treatment was continued until stabilisation or progression.

Concomitant supportive and toxicity-preventive treatments (corticosteroids, setrons, atropine and loperamide, haematopoietic-stimulating factors) were allowed.

2.4. Dose adjustment

Treatment adjustment was done as follow: at the first episode of grade 3/4 toxicity, treatment was interrupted until regression $\leq$ grade 2 for haematological toxicity and to grade 0 for gastrointestinal toxicity. Then chemotherapy was pursued with a 20% reduction of the original dose. A second episode of any grade 3/4 toxicity led to a 50% reduction of the original dose. Treatment was definitively stopped if a third episode grade 3/4 toxicity occurred. No dose escalation was permitted. Treatment was stopped if the patient did not recover grade 2 toxicity or within 3 weeks after the planned date of chemotherapy administration.

2.5. Follow-up assessment

The tumour responses were evaluated every 3 months including MRI or CT-scan measurements of the target lesions and were classified according to RECIST v1.0 criteria [13].

The relevant biomarkers were measured every 3 months if they had been elevated at baseline. Biological complete response was defined as normalisation of chromogranin A or other elevated biomarkers levels, partial response as >50% reduction, stabilisation as variation from 25% to 50%, and progression as a >25% increase.

2.6. Statistical methods

All analyses were performed according to intent-to-treat for all included patients, regardless of eligibility criteria and treatment received. The primary end point was the non-progression rate at 6 months defined as the number of patients free of progression 6 months after treatment initiation. Secondary end points

Table 1
Characteristics of the 20 patients with metastatic well-differentiated pancreatic endocrine carcinoma.

Characteristics	Value
Age (mean/SD) years	58.1 (12.3)
Men, *n* (%)	13 (65)
WHO performance status ≤2, *n* (%)	18 (90)
Metastase sites, *n* (%)	
Liver	19 (95)
Lungs	1 (5)
Lymph nodes	6 (30)
Peritoneum	1 (5)
Bones	3 (15)
Other	0 (0)
Functional tumour, *n* (%)	5 (25)
MEN 1, *n* (%)	2 (10)
Prior treatment, *n* (%)	
Chemotherapy	0 (0)
Chemoembolisation	0 (0)
External radiotherapy	1 (5)
Surgery	7 (35)
Somatostatin analogues	5 (25)
KI67 ≥ 15%, *n* (%)	3 (15)
Chromogranin A > normal, *n* (%)	12 (60)

SD: standard deviation, MEN 1: multiple endocrine neoplasia type 1.

months, progression-free survival (PFS), time-to-treatment failure (TTF), disease duration control, overall survival (OS) and safety.

PFS was defined as the time from inclusion until the date of first progression or death (any cause); TTF was defined as the time from inclusion until definitive treatment discontinuation because of progression, toxicity or other reasons; disease duration control was defined as the time interval between response or stability and 1st progression or tumour-related death. OS was defined as the time from inclusion until the date of death (any cause) or the last follow-up visit for a surviving patient. Progression rates were reported using frequency and percent with its 95% confidence intervals (CI). Continuous variables are given as using means ± standard deviation (SD) or medians (range). Survival times were estimated using the Kaplan–Meier method and described as medians [95% CI].

Follow-up was calculated using reverse Kaplan–Meier estimation and reported as medians.

Twenty patients had to be enrolled to use Fleming one-step design (5% unilateral alpha type-one error and 80% power) and the following hypotheses: H0 a non-progression rate at 6 months of 60% is no improvement and H1 a non-progression rate at 6 months of 85% is expected [16]. Fleming's decision rules were the following: if we observed 15 or fewer progression-free patients at 6 months, the treatment will be declared not an improvement; if we observed 16 or more progression-free patients at 6 months, the treatment will be declared promising.

Kurskal–Wallis was used to estimate the distribution of the Ki-67 index according to the best response.

3. Results

3.1. Patient's characteristics

A total of 20 patients from 6 French hospitals were included in the study between May 2004 and July 2005. Median follow-up was 31 months (95% CI 29–35). Patient characteristics at inclusion are summarised in Table 1. All were stage IV according to the TNM ENETS classification. The Ki 67 staining was available for 16 patients (insufficient amount of material in 4) and was ≤ 15% in 13 patients. Tumour was non-functional in 15 patients.

Four patients did not meet the major eligibility criteria: 3

Table 2
Maximal grade toxicity observed during FOLFIRI chemotherapy (NCI–CTC version 2.0).

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Overall	0	0	4 (20)	10 (50)	6 (30)
Haematological	3 (15)	4 (20)	8 (40)	2 (10)	3 (15)
Leucopenia	11 (55)	5 (25)	3 (15)	3 (15)	0
Neutropenia	6 (30)	5 (25)	4 (20)	2 (10)	3 (15)
Febrile neutropenia	19 (95)	0	1 (5)	0	0
Non haematological	0	2 (10)	5 (25)	10 (50)	3 (15)
Nausea	9 (45)	4 (20)	3 (15)	4 (20)	0
Vomiting	10 (50)	1 (5)	7 (35)	2 (10)	0
Mucositis	14 (70)	5 (25)	0	1 (5)	0

Results are reported as number of patients (%).

radiotherapy to the primary tumour, although that lesion was not used as a measurable target.

3.2. Treatment and its toxicity

All patients received at least 1 chemotherapy cycle. The median number of cycles was 12 [range 1–28]. Median treatment time was 5.3 [range 0–23] months. Eight patients had at least 1 treatment break (6 had 1 and 2 had 2 breaks). During chemotherapy, the WHO PS remained ≤2 for all the patients.

A majority of the patients (80%) experienced grade 3/4 toxicity during treatment (Table 2). However, only 6 (30%) patients had grade 4 toxicity: neutropenia for 3, thrombosis for 1, pain for 1 and rhabdomyolysis for 1; 1 patient had grade 2 febrile neutropenia. Treatment was stopped because of toxicity for 4 patients (20%), and dose was reduced for 5 (25%). Global haematologic grade 3–4 toxicity was 25%. No toxic death was recorded. Eight patients developed severe adverse events: pulmonary embolism for 2, melena and rhabdomyolysis for 1, severe diarrhoea with dehydratation for 2, diabetes decompensation for 2, and gastric perforation requiring surgery for 1.

3.3. Response and survival

The non-progression rate at 6 months was 80% (95% CI: 56–94%): stabilisation for 15 patients, objective response for 1, and disease progression in the remaining 4 (Table 3). Then 16 patients were free of progression at 6 months, meaning that regarding Fleming's decision rules, the non-progression rate was significantly higher than the H0 hypothesis of no improvement at 60% ($P = 0.05$).

The 24-month post-inclusion OS was 65% [40–82%] (Fig. 1), the median PFS was 9.1 [6.5–17.3] months (Fig. 2), the median TTF was 6.5 [3.1–15.5] months, the median disease control duration was 8.6 [3.0–24.8] months. It was calculated for 13 of the 20 patients because 2 patient's disease did not progress, 4 patients had tumour progression at the first evaluation, and the last patient's first tumour evaluation was made at 30 months of follow-up.

Twelve of the 16 patients with progressive disease received second-line therapy: chemotherapy for 9, and somatostatin analogue, chemoembolisation or Lipiocis treatment, for 1 each.

Table 3
Tumour responses according to time after starting treatment.

Date of evaluation	Objective response	Stable disease	Progression
6 months	1 (5%)	15 (75)	4 (20)
12 months	0	9 (45)	11 (55)
18 months	0	5 (25)	15 (75)
24 months	0	4 (20)	16 (80)

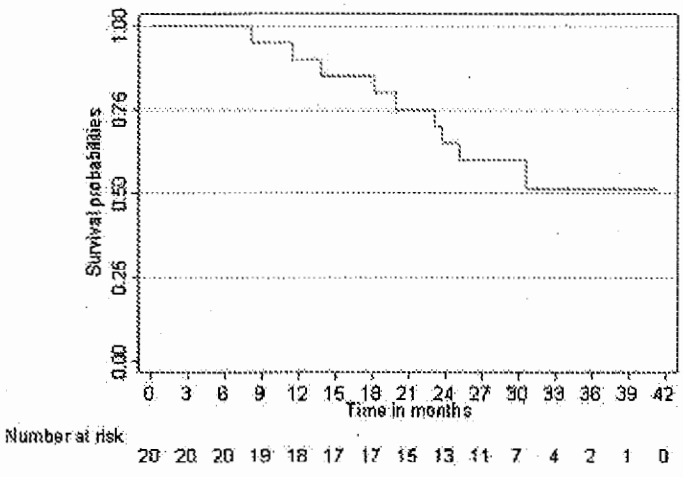

Fig. 1. Overall survival (Kaplan–Meier method).

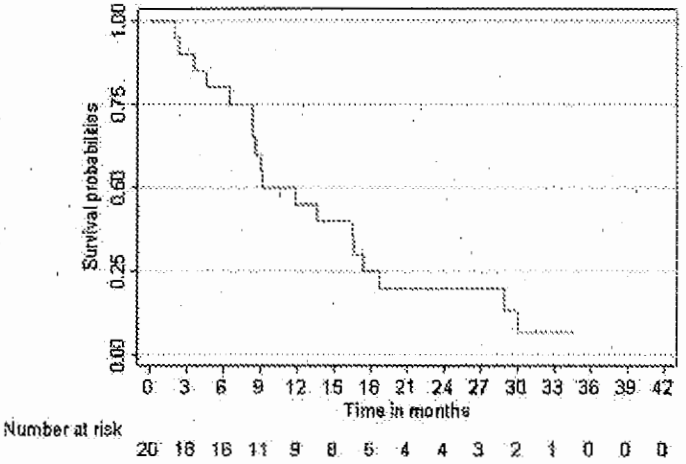

Fig. 2. Progression free survival (Kaplan–Meier method).

At the data cut-off, amongst the 20 patients, 9 died with progression, 9 were alive with progression and 2 were alive without progression.

Chromogranin A levels at baseline were elevated in 12 patients. Partial biological responses were observed in 3, progression in 9 and stability in 4. Radiological responses did not parallel the biological responses. The Ki-67 index, determined for 16 patients, was not predictive of tumour response since Ki-67 distribution did not differ according to the best response during treatment (Kruskal–Wallis $P = 0.29$).

4. Discussion

Progressive unresectable pancreatic NETs have limited treatment options and at the time this study was conducted no chemotherapy has proven high response rate. Moreover data on PFS, duration of stabilisation and OS are heterogeneous when they are available. Thus comparisons of data from these studies with data from the current study are difficult. Although our study included a small number of patients, it was prospective, all patients had histological proven well-differentiated endocrine carcinoma of the pancreas, all were chemotherapy-naive and all had documented disease progression according to the RECIST criteria during the six months preceding enrolment. In previously published studies, the non-progression rate at 6 months was 80% (56–94). Non progression rate is a marker of efficacy because stabililisation leads to improve survival [17–19].

The FOLFIRI regimen has been evaluated in only one previous study to treat endocrine tumours of various primary lesions in pretreated patients [12]. Both studies recorded only 1 objective response amongst their 20 enrolled patients. This result might be considered disappointing compared to the standard chemotherapy regimen (doxorubicin–streptozotocin) with 2 studies that found high response rates (69% and 36%) [8,9], although 2 others showed only 6% objective response rates [10,11], and compared to recent studies with other regimens that gave very enthusiastic results. The combination capecitabine–temozolomide as first-line chemotherapy in patients with advanced neuroendocrine pancreatic tumours gave 70% objective response rates and 27% stabilisation [20]. Two other recent studies performed in patients with different types of advanced neuroendocrine tumours with 5 fluorouracil–cisplatin–streptozotocine [21] and 5 fluorouracil–dacarbazine–epirubicin [22] showed 89–95% tumour control rates in the subgroup of patients with pancreatic tumours, with 38% and 58% objective response rates, respectively.

In our study the PFS was 9.1 months, longer than Ducreux et al.'s [12] (5 months) and within the range obtained with doxorubicin–streptozotocin (from 3.9 months [11] to 15.0 months [9]), but lower than that obtained with other recently evaluated regimens: 17 months to 18 months with 5 fluorouracil–doxorubicin–streptozotocin [23], 5 fluorouracil–dacarbazine–epirubicin [22] and capecitabine–temozolomide [20].

Two recent large studies showed that the targeted therapies sunitinib and everolimus significantly increased PFS as compared to placebo in patients with advanced well-differentiated endocrine carcinomas of the pancreas [19,24]. Although the comparison of our data with the latter studies is debatable, FOLFIRI gave similar results for PFS, 9.1 months versus 11.4 months [19] and 11 months [24], respectively.

Haematological toxicity, especially grade 3/4 neutropenia (25%) was similar to haematological toxicity recorded with doxorubicin–streptozotocin by Delaunoit et al. [9] (24%) and less than that observed in patients given the same regimen for colon cancer [25] (60%) and in the study by Ducreux et al. [12] (40%), but digestive toxicity was similar. We can speculate that the haematological tolerability was better because our patients did not receive chemotherapy previously. Toxicity of targeted therapies is very different [19,24]. The FOLFIRI regimen is easy to use: half-day hospitalisation versus 5 days for the doxorubicin–streptozotocin regimen, and does not lead to renal and cardiac toxicities. Pertinently, the toxicity profile, particularly haematological toxicity, and FOLFIRI regimen efficacy could be improved by the determination of the drug-metabolising enzyme uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) polymorphism [26].

FOLFIRI regimen efficacy against colon cancer has been improved by combining it with bevacizumab [27]. Endocrine tumours are known to be highly vascular and to overexpress vascular endothelium growth factor (VEGF) [28,29]. So the rationale for using VEGF-pathway inhibitors to treat endocrine carcinomas is logical. Some encouraging results have also been obtained with bevacizumab against carcinoid tumours [30]. The combination of FOLFIRI regimen and bevacizumab should be tested against unresectable metastatic endocrine carcinomas, as it has been done with other chemotherapies with encouraging results [31,32].

Our patients had few treatment breaks, perhaps because of the low rate of objective responses or because these time-outs were a novel concept in chemotherapy and had not been applied by

The main weaknesses of the study concerns Ki67 indexes. It was not possible to measure it in 4 patients because of low amount of material and it was above the predetermined threshold in 3 patients. We cannot exclude that it might have influenced results. However, all tumours were well-differentiated. Ki67 has not been measured or taken into account in the most recently published studies on chemotherapy [20] and on targeted therapies [19,24], probably because its determination has been considered a standard only recently [15]. Furthermore the low amount of tissue material is a frequent drawback. Five patients previously received somatostatin analogues. It has been recently shown in the randomised placebo-controlled PROMID study that octreotide has a significant antitumour effect for well-differentiated endocrine carcinomas of the intestine [33]. Although this effect might also exist for well-differentiated endocrine tumours of the pancreas, it is much improbable that it has influenced the results of our study.

In conclusion, the FOLFIRI regimen induced stabilisation in most patients with progressive, chemotherapy-naive, well-differentiated endocrine carcinomas of the pancreas but only 1 objective response. It can be done on ambulatory hospitalisation. The toxicity profile can be improved by determination of the drug metabolising enzyme. This regimen could be an alternative to other chemotherapies or targeted therapies as a first-line therapy should the other drugs be contraindicated. Combination of targeted therapies and chemotherapies should be further evaluated, FOLFIRI regimen could be a good option.

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Conflict of interest statement

Hedia Brixi-Benmansour, Jean-Louis Jouve, Franck Bonnetain, Bruno Landi, Olivia Hentic, Laurent Bedenne: no conflict of interest that could be perceived as prejudicing the impartiality of the research reported; Guillaume Cadiot and Emmanuel Mitry: fees from Pfizer.

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CAMPTOSAR®
irinotecan hydrochloride injection

WARNINGS

CAMPTOSAR Injection should be administered only under the supervision of a physician who is experienced in the use of cancer chemotherapeutic agents. Appropriate management of complications is possible only when adequate diagnostic and treatment facilities are readily available.

CAMPTOSAR can induce both early and late forms of diarrhea that appear to be mediated by different mechanisms. Both forms of diarrhea may be severe. Early diarrhea (occurring during or shortly after infusion of CAMPTOSAR) may be accompanied by cholinergic symptoms of rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, and intestinal hyperperistalsis that can cause abdominal cramping. Early diarrhea and other cholinergic symptoms may be prevented or ameliorated by atropine (see PRECAUTIONS, General). Late diarrhea (generally occurring more than 24 hours after administration of CAMPTOSAR) can be life threatening since it may be prolonged and may lead to dehydration, electrolyte imbalance, or sepsis. Late diarrhea should be treated promptly with loperamide. Patients with diarrhea should be carefully monitored and given fluid and electrolyte replacement if they become dehydrated, or antibiotic therapy if they develop ileus, fever, or severe neutropenia (see WARNINGS). Administration of CAMPTOSAR should be interrupted and subsequent doses reduced if severe diarrhea occurs (see DOSAGE AND ADMINISTRATION).

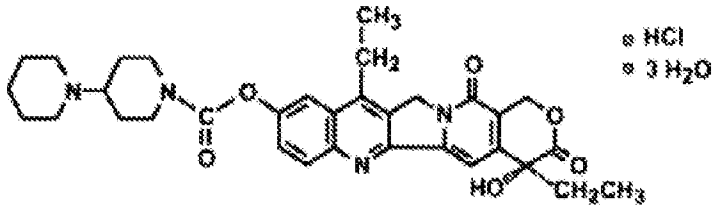
Severe myelosuppression may occur (see WARNINGS).

DESCRIPTION

CAMPTOSAR Injection (irinotecan hydrochloride injection) is an antineoplastic agent of the topoisomerase I inhibitor class. Irinotecan hydrochloride was clinically investigated as CPT-11.

CAMPTOSAR is supplied as a sterile, pale yellow, clear, aqueous solution. It is available in two single-dose sizes: 2 mL-fill vials contain 40 mg irinotecan hydrochloride and 5 mL-fill vials contain 100 mg irinotecan hydrochloride. Each milliliter of solution contains 20 mg of irinotecan hydrochloride (on the basis of the trihydrate salt), 45 mg of sorbitol NF powder, and 0.9 mg of lactic acid, USP. The pH of the solution has been adjusted to 3.5 (range, 3.0 to 3.8) with sodium hydroxide or hydrochloric acid. CAMPTOSAR is intended for dilution with 5% Dextrose Injection, USP (D5W), or 0.9% Sodium Chloride Injection, USP, prior to intravenous infusion. The preferred diluent is 5% Dextrose Injection, USP.

Irinotecan hydrochloride is a semisynthetic derivative of camptothecin, an alkaloid extract from plants such as *Camptotheca acuminata*. The chemical name is *(S)*-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1*H*-pyrano[3',4':6,7]-indolizino[1,2-b]quinolin-9-yl-[1,4'-bipiperidine]-1'-carboxylate, monohydrochloride, trihydrate. Its structural formula is as follows:



Irinotecan Hydrochloride

Irinotecan hydrochloride is a pale yellow to yellow crystalline powder, with the empirical formula $C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3H_2O$ and a molecular weight of 677.19. It is slightly soluble in water and organic solvents.

CLINICAL PHARMACOLOGY

Irinotecan is a derivative of camptothecin. Camptothecins interact specifically with the enzyme topoisomerase I which relieves torsional strain in DNA by inducing reversible single-strand breaks. Irinotecan and its active metabolite SN-38 bind to the topoisomerase I-DNA complex and prevent religation of these single-strand breaks. Current research suggests that the cytotoxicity of irinotecan is due to double-strand DNA damage produced during DNA synthesis when replication enzymes interact with the ternary complex formed by topoisomerase I, DNA, and either irinotecan or SN-38. Mammalian cells cannot efficiently repair these double-strand breaks.

Irinotecan serves as a water-soluble precursor of the lipophilic metabolite SN-38. SN-38 is formed from irinotecan by carboxylesterase-mediated cleavage of the carbamate bond between the camptothecin moiety and the dipiperidino side chain. SN-38 is approximately 1000 times as potent as irinotecan as an inhibitor of topoisomerase I purified from human and rodent tumor cell lines. In vitro cytotoxicity assays show that the potency of SN-38 relative to irinotecan varies from 2- to 2000-fold. However, the plasma area under the concentration versus time curve (AUC) values for SN-38 are 2% to 8% of irinotecan and SN-38 is 95% bound to plasma proteins compared to approximately 50% bound to plasma proteins for irinotecan (see Pharmacokinetics). The precise contribution of SN-38 to the activity of CAMPTOSAR is thus unknown. Both irinotecan and SN-38 exist in an active lactone form and an inactive hydroxy acid anion form. A pH-dependent equilibrium exists between the two forms such that an acid pH promotes the formation of the lactone, while a more basic pH favors the hydroxy acid anion form.

Administration of irinotecan has resulted in antitumor activity in mice bearing cancers of rodent origin and in human carcinoma xenografts of various histological types.

Pharmacokinetics

After intravenous infusion of irinotecan in humans, irinotecan plasma concentrations decline in a multiexponential manner, with a mean terminal elimination half-life of about 6 to 12 hours. The mean terminal elimination half-life of the active metabolite SN-38 is about 10 to 20 hours. The half-lives of the lactone (active) forms of irinotecan and SN-38 are similar

to those of total irinotecan and SN-38, as the lactone and hydroxy acid forms are in equilibrium.

Over the recommended dose range of 50 to 350 mg/m^2, the AUC of irinotecan increases linearly with dose; the AUC of SN-38 increases less than proportionally with dose. Maximum concentrations of the active metabolite SN-38 are generally seen within 1 hour following the end of a 90-minute infusion of irinotecan. Pharmacokinetic parameters for irinotecan and SN-38 following a 90-minute infusion of irinotecan at dose levels of 125 and 340 mg/m^2 determined in two clinical studies in patients with solid tumors are summarized in Table 1:

Table 1. Summary Of Mean (± Standard Deviation) Irinotecan And SN-38 Pharmacokinetic Parameters In Patients With Solid Tumors

Dose (mg/m^2)	Irinotecan					SN-38		
	C_{max} (ng/mL)	AUC_{0-24} (ng•h/mL)	$t_{½}$ (h)	V_z (L/m^2)	CL (L/h/m^2)	C_{max} (ng/mL)	AUC_{0-24} (ng•h/mL)	$t_{½}$ (h)
125 (N=64)	1,660 ± 797	10,200 ± 3,270	5.8[a] ± 0.7	110 ± 48.5	13.3 ± 6.01	26.3 ± 11.9	229 ± 108	10.4[a] ± 3.1
340 (N=6)	3,392 ± 874	20,604 ± 6,027	11.7[b] ± 1.0	234 ± 69.6	13.9 ± 4.00	56.0 ± 28.2	474 ± 245	21.0[b] ± 4.3

C_{max} - Maximum plasma concentration
AUC_{0-24} - Area under the plasma concentration-time curve from time 0 to 24 hours after the end of the 90-minute infusion
$t_{½}$ - Terminal elimination half-life
V_z - Volume of distribution of terminal elimination phase
CL - Total systemic clearance
[a] Plasma specimens collected for 24 hours following the end of the 90-minute infusion.
[b] Plasma specimens collected for 48 hours following the end of the 90-minute infusion. Because of the longer collection period, these values provide a more accurate reflection of the terminal elimination half-lives of irinotecan and SN-38.

Irinotecan exhibits moderate plasma protein binding (30% to 68% bound). SN-38 is highly bound to human plasma proteins (approximately 95% bound). The plasma protein to which irinotecan and SN-38 predominantly binds is albumin.

Metabolism and Excretion: The metabolic conversion of irinotecan to the active metabolite SN-38 is mediated by carboxylesterase enzymes and primarily occurs in the liver. SN-38 subsequently undergoes conjugation to form a glucuronide metabolite. SN-38 glucuronide had 1/50 to 1/100 the activity of SN-38 in cytotoxicity assays using two cell lines in vitro. The disposition of irinotecan has not been fully elucidated in humans. The urinary excretion of irinotecan is 11% to 20%; SN-38, <1%; and SN-38 glucuronide, 3%. The cumulative biliary and urinary excretion of irinotecan and its metabolites (SN-38 and SN-38 glucuronide) over a period of 48 hours following administration of irinotecan in two patients ranged from approximately 25% (100 mg/m^2) to 50% (300 mg/m^2).

Pharmacokinetics in Special Populations

Geriatric: In studies using the weekly schedule, the terminal half-life of irinotecan was 6.0 hours in patients who were 65 years or older and 5.5 hours in patients younger than 65 years. Dose-normalized AUC_{0-24} for SN-38 in patients who were at least 65 years of age

was 11% higher than in patients younger than 65 years. No change in the starting dose is recommended for geriatric patients receiving the weekly dosage schedule of irinotecan. The pharmacokinetics of irinotecan given once every 3 weeks has not been studied in the geriatric population; a lower starting dose is recommended in patients 70 years or older based on clinical toxicity experience with this schedule (see DOSAGE AND ADMINISTRATION).
Pediatric: Information regarding the pharmacokinetics of irinotecan is not available.
Gender: The pharmacokinetics of irinotecan do not appear to be influenced by gender.
Race: The influence of race on the pharmacokinetics of irinotecan has not been evaluated.
Hepatic Insufficiency: The influence of hepatic insufficiency on the pharmacokinetic characteristics of irinotecan and its metabolites has not been formally studied. Among patients with known hepatic tumor involvement (a majority of patients), irinotecan and SN-38 AUC values were somewhat higher than values for patients without liver metastases (see PRECAUTIONS).
Renal Insufficiency: The influence of renal insufficiency on the pharmacokinetics of irinotecan has not been evaluated.

Drug-Drug Interactions

In a phase 1 clinical study involving irinotecan, 5-fluorouracil (5-FU), and leucovorin (LV) in 26 patients with solid tumors, the disposition of irinotecan was not substantially altered when the drugs were co-administered. Although the C_{max} and AUC_{0-24} of SN-38, the active metabolite, were reduced (by 14% and 8%, respectively) when irinotecan was followed by 5-FU and LV administration compared with when irinotecan was given alone, this sequence of administration was used in the combination trials and is recommended (see DOSAGE AND ADMINISTRATION). Formal in vivo or in vitro drug interaction studies to evaluate the influence of irinotecan on the disposition of 5-FU and LV have not been conducted.

Possible pharmacokinetic interactions of CAMPTOSAR with other concomitantly administered medications have not been formally investigated.

CLINICAL STUDIES

Irinotecan has been studied in clinical trials in combination with 5-fluorouracil (5-FU) and leucovorin (LV) and as a single agent (see DOSAGE AND ADMINISTRATION). When given as a component of combination-agent treatment, irinotecan was either given with a weekly schedule of bolus 5-FU/LV or with an every-2-week schedule of infusional 5-FU/LV. Weekly and a once-every-3-week dosage schedules were used for the single-agent irinotecan studies. Clinical studies of combination and single-agent use are described below.

First-Line Therapy in Combination with 5-FU/LV for the Treatment of Metastatic Colorectal Cancer

Two phase 3, randomized, controlled, multinational clinical trials support the use of CAMPTOSAR Injection as first-line treatment of patients with metastatic carcinoma of the colon or rectum. In each study, combinations of irinotecan with 5-FU and LV were compared with 5-FU and LV alone. Study 1 compared combination irinotecan/bolus 5-FU/LV therapy given weekly with a standard bolus regimen of 5-FU/LV alone given daily for 5 days every 4 weeks; an irinotecan-alone treatment arm given on a weekly schedule was

also included. Study 2 evaluated two different methods of administering infusional 5-FU/LV, with or without irinotecan. In both studies, concomitant medications such as antiemetics, atropine, and loperamide were given to patients for prophylaxis and/or management of symptoms from treatment. In Study 2, a 7-day course of fluoroquinolone antibiotic prophylaxis was given in patients whose diarrhea persisted for greater than 24 hours despite loperamide or if they developed a fever in addition to diarrhea. Treatment with oral fluoroquinolone was also initiated in patients who developed an absolute neutrophil count (ANC) < 500/mm^3, even in the absence of fever or diarrhea. Patients in both studies also received treatment with intravenous antibiotics if they had persistent diarrhea or fever or if ileus developed.

In both studies, the combination of irinotecan/5-FU/LV therapy resulted in significant improvements in objective tumor response rates, time to tumor progression, and survival when compared with 5-FU/LV alone. These differences in survival were observed in spite of second-line therapy in a majority of patients on both arms, including crossover to irinotecan-containing regimens in the control arm. Patient characteristics and major efficacy results are shown in Table 2.

Table 2. Combination Dosage Schedule: Study Results

	Study 1			Study 2	
	Irinotecan + Bolus 5-FU/LV weekly x 4 q 6 weeks	**Bolus 5-FU/LV daily x 5 q 4 weeks**	**Irinotecan weekly x 4 q 6 weeks**	**Irinotecan + Infusional 5-FU/LV**	**Infusional 5-FU/LV**
Number of Patients	231	226	226	198	187
Demographics and Treatment Administration					
Female/Male (%)	34/65	45/54	35/64	33/67	47/53
Median Age in years (range)	62 (25-85)	61 (19-85)	61 (30-87)	62 (27-75)	59 (24-75)
Performance Status (%)					
0	39	41	46	51	51
1	46	45	46	42	41
2	15	13	8	7	8
Primary Tumor (%)					
Colon	81	85	84	55	65
Rectum	17	14	15	45	35
Median Time from Diagnosis to Randomization (months, range)	1.9 (0-161)	1.7 (0-203)	1.8 (0.1-185)	4.5 (0-88)	2.7 (0-104)
Prior Adjuvant 5-FU Therapy (%)					
No	89	92	90	74	76
Yes	11	8	10	26	24
Median Duration of Study Treatment[a] (months)	5.5	4.1	3.9	5.6	4.5
Median Relative Dose Intensity (%)[a]					
Irinotecan	72	--	75	87	--
5-FU	71	86	--	86	93
Efficacy Results					
Confirmed Objective Tumor Response Rate[b] (%)	39	21 ($p<0.0001$)[c]	18	35	22 ($p<0.005$)[c]
Median Time to Tumor Progression[d] (months)	7.0	4.3 ($p=0.004$)[d]	4.2	6.7	4.4 ($p<0.001$)[d]
Median Survival (months)	14.8	12.6 ($p<0.05$)[d]	12.0	17.4	14.1 ($p<0.05$)[d]

[a] Study 1: N=225 (irinotecan/5-FU/LV), N=219 (5-FU/LV), N=223 (irinotecan)
Study 2: N=199 (irinotecan/5-FU/LV), N=186 (5-FU/LV)

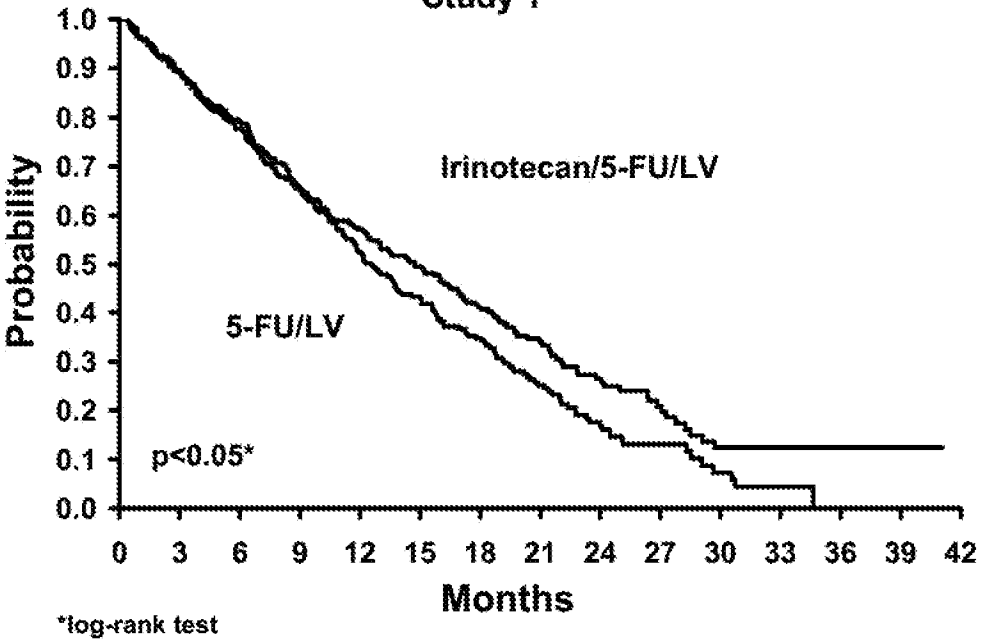
[b] Confirmed ≥ 4 to 6 weeks after first evidence of objective response

[c] Chi-square test

[d] Log-rank test

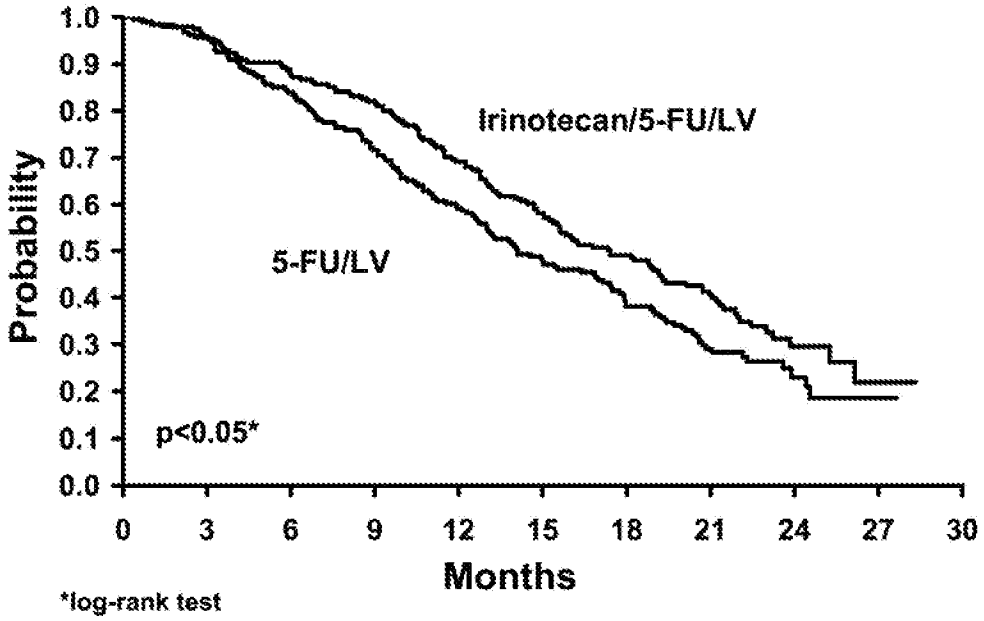
Improvement was noted with irinotecan-based combination therapy relative to 5-FU/LV when response rates and time to tumor progression were examined across the following demographic and disease-related subgroups (age, gender, ethnic origin, performance status, extent of organ involvement with cancer, time from diagnosis of cancer, prior adjuvant therapy, and baseline laboratory abnormalities). Figures 1 and 2 illustrate the Kaplan-Meier survival curves for the comparison of irinotecan/5-FU/LV versus 5-FU/LV in Studies 1 and 2, respectively.

Figure 1. Survival
First-Line Irinotecan/5-FU/LV vs 5-FU/LV
Study 1



*log-rank test

Figure 2. Survival
First-Line Irinotecan/5-FU/LV vs 5-FU/LV
Study 2



Second-Line Treatment for Recurrent or Progressive Metastatic Colorectal Cancer After 5-FU-Based Treatment

Weekly Dosage Schedule

Data from three open-label, single-agent, clinical studies, involving a total of 304 patients in 59 centers, support the use of CAMPTOSAR in the treatment of patients with metastatic cancer of the colon or rectum that has recurred or progressed following treatment with 5-FU-based therapy. These studies were designed to evaluate tumor response rate and do not provide information on actual clinical benefit, such as effects on survival and disease-related symptoms. In each study, CAMPTOSAR was administered in repeated 6-week cycles consisting of a 90-minute intravenous infusion once weekly for 4 weeks, followed by a 2-week rest period. Starting doses of CAMPTOSAR in these trials were 100, 125, or 150 mg/m^2, but the 150-mg/m^2 dose was poorly tolerated (due to unacceptably high rates of grade 4 late diarrhea and febrile neutropenia). Study 1 enrolled 48 patients and was conducted by a single investigator at several regional hospitals. Study 2 was a multicenter study conducted by the North Central Cancer Treatment Group. All 90 patients enrolled in Study 2 received a starting dose of 125 mg/m^2. Study 3 was a multicenter study that enrolled 166 patients from 30 institutions. The initial dose in Study 3 was 125 mg/m^2 but was reduced to 100 mg/m^2 because the toxicity seen at the 125-mg/m^2 dose was perceived to be greater than that seen in previous studies. All patients in these studies had metastatic colorectal

cancer, and the majority had disease that recurred or progressed following a 5-FU-based regimen administered for metastatic disease. The results of the individual studies are shown in Table 3.

Table 3. Weekly Dosage Schedule: Study Results

	Study			
	1	2	3	
Number of Patients	48	90	64	102
Starting Dose (mg/m^2/wk x 4)	125[a]	125	125	100
Demographics and Treatment Administration				
Female/Male (%)	46/54	36/64	50/50	51/49
Median Age in years (range)	63 (29-78)	63 (32-81)	61 (42-84)	64 (25-84)
Ethnic Origin (%)				
White	79	96	81	91
African American	12	4	11	5
Hispanic	8	0	8	2
Oriental/Asian	0	0	0	2
Performance Status (%)				
0	60	38	59	44
1	38	48	33	51
2	2	14	8	5
Primary Tumor (%)				
Colon	100	71	89	87
Rectum	0	29	11	8
Unknown	0	0	0	5
Prior 5-FU Therapy (%)				
For Metastatic Disease	81	66	73	68
≤ 6 months after Adjuvant	15	7	27	28
> 6 months after Adjuvant	2	16	0	2
Classification Unknown	2	12	0	3
Prior Pelvic/Abdominal Irradiation (%)				
Yes	3	29	0	0
Other	0	9	2	4
None	97	62	98	96
Duration of Treatment with CAMPTOSAR (median, months)	5	4	4	3
Relative Dose Intensity[b] (median %)	74	67	73	81
Efficacy				
Confirmed Objective Response Rate (%)[c]	21	13	14	9
(95% CI)	(9.3 - 32.3)	(6.3 - 20.4)	(5.5 - 22.6)	(3.3 - 14.3)
Time to Response (median, months)	2.6	1.5	2.8	2.8
Response Duration (median, months)	6.4	5.9	5.6	6.4
Survival (median, months)	10.4	8.1	10.7	9.3
1-Year Survival (%)	46	31	45	43

[a] Nine patients received 150 mg/m^2 as a starting dose; two (22.2%) responded to CAMPTOSAR.

[b] Relative dose intensity for CAMPTOSAR based on planned dose intensity of 100, 83.3, and 66.7 mg/m^2/wk corresponding with 150, 125, and 100 mg/m^2 starting doses, respectively.

[c] Confirmed ≥4 to 6 weeks after first evidence of objective response.

In the intent-to-treat analysis of the pooled data across all three studies, 193 of the 304 patients began therapy at the recommended starting dose of 125 mg/m^2. Among these 193 patients, 2 complete and 27 partial responses were observed, for an overall response rate of 15.0% (95% Confidence Interval [CI], 10.0% to 20.1%) at this starting dose. A considerably lower response rate was seen with a starting dose of 100 mg/m^2. The majority of responses were observed within the first two cycles of therapy, but responses did occur in later cycles of treatment (one response was observed after the eighth cycle). The median response duration for patients beginning therapy at 125 mg/m^2 was 5.8 months (range, 2.6 to 15.1 months). Of the 304 patients treated in the three studies, response rates to CAMPTOSAR were similar in males and females and among patients older and younger than 65 years. Rates were also similar in patients with cancer of the colon or cancer of the rectum and in patients with single and multiple metastatic sites. The response rate was 18.5% in patients with a performance status of 0 and 8.2% in patients with a performance status of 1 or 2. Patients with a performance status of 3 or 4 have not been studied. Over half of the patients responding to CAMPTOSAR had not responded to prior 5-FU. Patients who had received previous irradiation to the pelvis responded to CAMPTOSAR at approximately the same rate as those who had not previously received irradiation.

Once-Every-3-Week Dosage Schedule

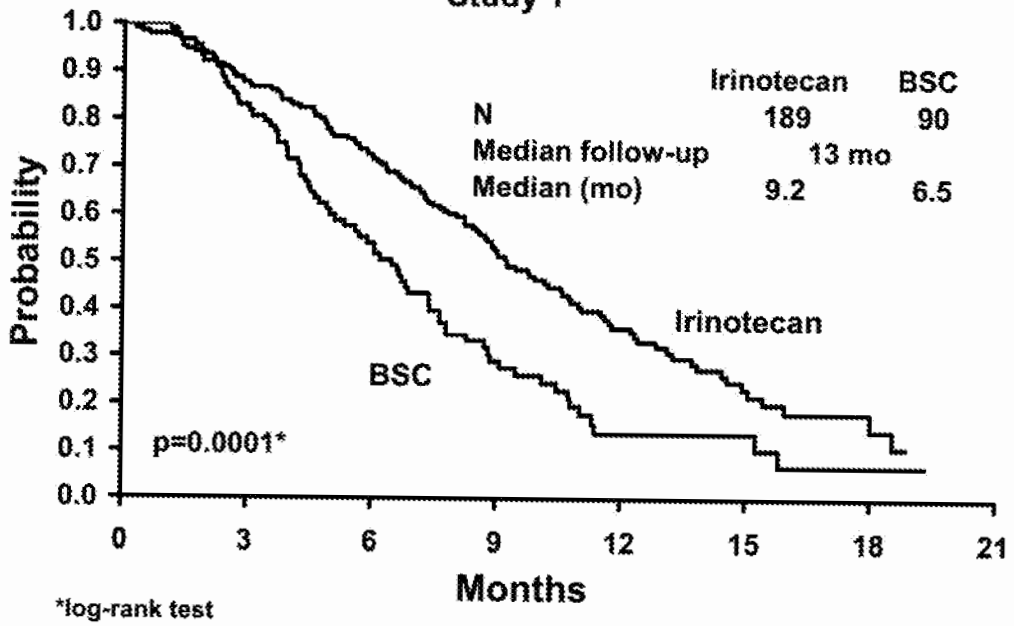
Single-Arm Studies: Data from an open-label, single-agent, single-arm, multicenter, clinical study involving a total of 132 patients support a once every-3-week dosage schedule of irinotecan in the treatment of patients with metastatic cancer of the colon or rectum that recurred or progressed following treatment with 5-FU. Patients received a starting dose of 350 mg/m^2 given by 30-minute intravenous infusion once every 3 weeks. Among the 132 previously treated patients in this trial, the intent-to-treat response rate was 12.1% (95% CI, 7.0% to 18.1%).

Randomized Trials: Two multicenter, randomized, clinical studies further support the use of irinotecan given by the once-every-3-week dosage schedule in patients with metastatic colorectal cancer whose disease has recurred or progressed following prior 5-FU therapy. In the first study, second-line irinotecan therapy plus best supportive care was compared with best supportive care alone. In the second study, second-line irinotecan therapy was compared with infusional 5-FU-based therapy. In both studies, irinotecan was administered intravenously at a starting dose of 350 mg/m^2 over 90 minutes once every 3 weeks. The starting dose was 300 mg/m^2 for patients who were 70 years and older or who had a performance status of 2. The highest total dose permitted was 700 mg. Dose reductions and/or administration delays were permitted in the event of severe hematologic and/or nonhematologic toxicities while on treatment. Best supportive care was provided to patients in both arms of Study 1 and included antibiotics, analgesics, corticosteroids, transfusions, psychotherapy, or any other symptomatic therapy as clinically indicated. In both studies, concomitant medications such as antiemetics, atropine, and loperamide were given to patients for prophylaxis and/or management of symptoms from treatment. If late diarrhea persisted for greater than 24 hours despite loperamide, a 7-day course of fluoroquinolone antibiotic prophylaxis was given. Patients in the control arm of the second study received one of the following 5-FU regimens: (1) LV, 200 mg/m^2 IV over 2 hours; followed by 5-FU, 400 mg/m^2 IV bolus; followed by 5-FU, 600 mg/m^2 continuous IV infusion over 22 hours on days

1 and 2 every 2 weeks; (2) 5-FU, 250 to 300 mg/m^2 /day protracted continuous IV infusion until toxicity; (3) 5-FU, 2.6 to 3 g/m^2 IV over 24 hours every week for 6 weeks with or without LV, 20 to 500 mg/m^2 /day every week IV for 6 weeks with 2-week rest between cycles. Patients were to be followed every 3 to 6 weeks for 1 year.

A total of 535 patients were randomized in the two studies at 94 centers. The primary endpoint in both studies was survival. The studies demonstrated a significant overall survival advantage for irinotecan compared with best supportive care (p=0.0001) and infusional 5-FU-based therapy (p=0.035) as shown in Figures 3 and 4. In Study 1, median survival for patients treated with irinotecan was 9.2 months compared with 6.5 months for patients receiving best supportive care. In Study 2, median survival for patients treated with irinotecan was 10.8 months compared with 8.5 months for patients receiving infusional 5-FU-based therapy. Multiple regression analyses determined that patients' baseline characteristics also had a significant effect on survival. When adjusted for performance status and other baseline prognostic factors, survival among patients treated with irinotecan remained significantly longer than in the control populations (p=0.001 for Study 1 and p=0.017 for Study 2). Measurements of pain, performance status, and weight loss were collected prospectively in the two studies; however, the plan for the analysis of these data was defined retrospectively. When comparing irinotecan with best supportive care in Study 1, this analysis showed a statistically significant advantage for irinotecan, with longer time to development of pain (6.9 months versus 2.0 months), time to performance status deterioration (5.7 months versus 3.3 months), and time to > 5% weight loss (6.4 months versus 4.2 months). Additionally, 33.3% (33/99) of patients with a baseline performance status of 1 or 2 showed an improvement in performance status when treated with irinotecan versus 11.3% (7/62) of patients receiving best supportive care (p=0.002). Because of the inclusion of patients with non-measurable disease, intent-to-treat response rates could not be assessed.

Figure 3. Survival
Second-Line Irinotecan vs Best Supportive Care (BSC)
Study 1



*log-rank test

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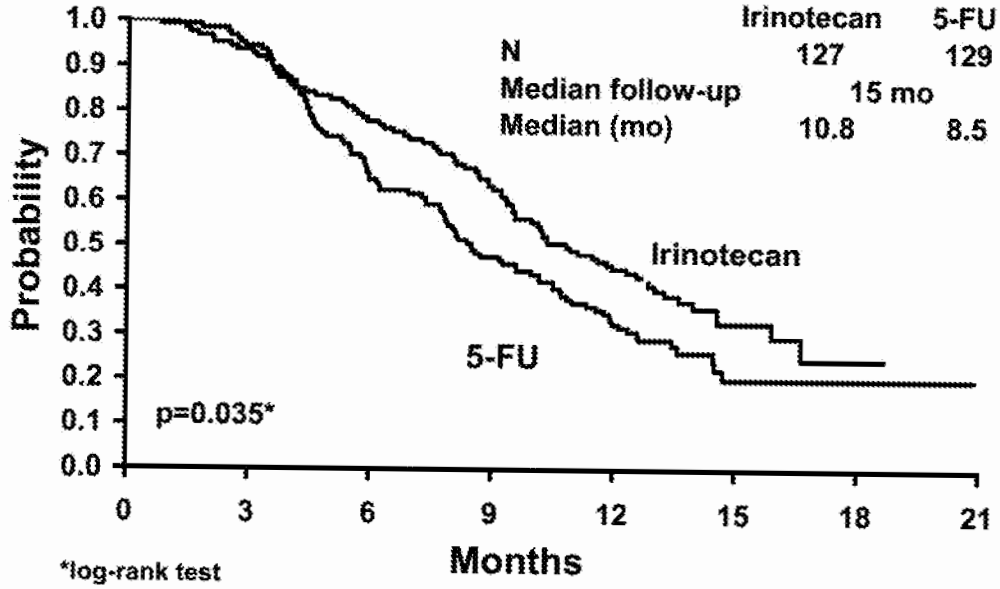
Figure 4. Survival
Second-Line Irinotecan vs Infusional 5-FU
Study 2



*log-rank test

Table 4. Once-Every-3-Week Dosage Schedule: Study Results

	Study 1		Study 2	
	Irinotecan	BSC[a]	Irinotecan	5-FU
Number of Patients	189	90	127	129
Demographics and Treatment Administration				
Female/Male (%)	32/68	42/58	43/57	35/65
Median Age in years (range)	59 (22-75)	62 (34-75)	58 (30-75)	58 (25-75)
Performance Status (%)				
0	47	31	58	54
1	39	46	35	43
2	14	23	8	3
Primary Tumor (%)				
Colon	55	52	57	62
Rectum	45	48	43	38
Prior 5-FU Therapy (%)				
For Metastatic Disease	70	63	58	68
As Adjuvant Treatment	30	37	42	32
Prior Irradiation (%)	26	27	18	20
Duration of Study Treatment (median, months) (Log-rank test)	4.1	--	4.2 (p=0.02)	2.8
Relative Dose Intensity (median %)[b]	94	--	95	81-99
Survival				
Survival (median, months) (Log-rank test)	9.2 (p=0.0001)	6.5	10.8 (p=0.035)	8.5

[a] BSC = best supportive care

[b] Relative dose intensity for irinotecan based on planned dose intensity of 116.7 and 100 mg/m^2/wk corresponding with 350 and 300 mg/m^2 starting doses, respectively.

In the two randomized studies, the EORTC QLQ-C30 instrument was utilized. At the start of each cycle of therapy, patients completed a questionnaire consisting of 30 questions, such as "Did pain interfere with daily activities?" (1 = Not at All, to 4 = Very Much) and "Do you have any trouble taking a long walk?" (Yes or No). The answers from the 30 questions were converted into 15 subscales, that were scored from 0 to 100, and the global health status subscale that was derived from two questions about the patient's sense of general well being in the past week. In addition to the global health status subscale, there were five functional (i.e., cognitive, emotional, social, physical, role) and nine symptom (i.e., fatigue, appetite loss, pain assessment, insomnia, constipation, dyspnea, nausea/vomiting, financial impact, diarrhea) subscales. The results as summarized in Table 5 are based on patients' worst post-baseline scores. In Study 1, a multivariate analysis and univariate analyses of the individual subscales were performed and corrected for multivariate testing. Patients receiving irinotecan reported significantly better results for the global health status, on two of five functional subscales, and on four of nine symptom subscales. As expected, patients receiving irinotecan noted significantly more diarrhea than those receiving best supportive care. In Study 2, the multivariate analysis on all 15 subscales did not indicate a statistically significant difference between irinotecan and infusional 5-FU.

Table 5. EORTC QLQ-C30: Mean Worst Post-Baseline Score[a]

QLQ-C30 Subscale	Study 1			Study 2		
	Irinotecan	BSC	p-value	Irinotecan	5-FU	p-value
Global Health Status	47	37	0.03	53	52	0.9
Functional Scales						
Cognitive	77	68	0.07	79	83	0.9
Emotional	68	64	0.4	64	68	0.9
Social	58	47	0.06	65	67	0.9
Physical	60	40	0.0003	66	66	0.9
Role	53	35	0.02	54	57	0.9
Symptom Scales						
Fatigue	51	63	0.03	47	46	0.9
Appetite Loss	37	57	0.0007	35	38	0.9
Pain Assessment	41	56	0.009	38	34	0.9
Insomnia	39	47	0.3	39	33	0.9
Constipation	28	41	0.03	25	19	0.9
Dyspnea	31	40	0.2	25	24	0.9
Nausea/Vomiting	27	29	0.5	25	16	0.09
Financial Impact	22	26	0.5	24	15	0.3
Diarrhea	32	19	0.01	32	22	0.2

[a]For the five functional subscales and global health status subscale, higher scores imply better functioning, whereas, on the nine symptom subscales, higher scores imply more severe symptoms. The subscale scores of each patient were collected at each visit until the patient dropped out of the study.

INDICATIONS AND USAGE

CAMPTOSAR Injection is indicated as a component of first-line therapy in combination with 5-fluorouracil and leucovorin for patients with metastatic carcinoma of the colon or rectum. CAMPTOSAR is also indicated for patients with metastatic carcinoma of the colon or rectum whose disease has recurred or progressed following initial fluorouracil-based therapy.

CONTRAINDICATIONS

CAMPTOSAR Injection is contraindicated in patients with a known hypersensitivity to the drug.

WARNINGS

General

Outside of a well-designed clinical study, CAMPTOSAR Injection should not be used in combination with the "Mayo Clinic" regimen of 5-FU/LV (administration for 4-5 consecutive days every 4 weeks) because of reports of increased toxicity, including toxic deaths. CAMPTOSAR should be used as recommended (see DOSAGE AND ADMINISTRATION, Table 10).

In patients receiving either irinotecan/5-FU/LV or 5-FU/LV in the clinical trials, higher rates of hospitalization, neutropenic fever, thromboembolism, first-cycle treatment discontinuation, and early deaths were observed in patients with a baseline performance status of 2 than in patients with a baseline performance status of 0 or 1.

Diarrhea

CAMPTOSAR can induce both early and late forms of diarrhea that appear to be mediated by different mechanisms. Early diarrhea (occurring during or shortly after infusion of CAMPTOSAR) is cholinergic in nature. It is usually transient and only infrequently is severe. It may be accompanied by symptoms of rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, and intestinal hyperperistalsis that can cause abdominal cramping. Early diarrhea and other cholinergic symptoms may be prevented or ameliorated by administration of atropine (see PRECAUTIONS, General, for dosing recommendations for atropine).

Late diarrhea (generally occurring more than 24 hours after administration of CAMPTOSAR) can be life threatening since it may be prolonged and may lead to dehydration, electrolyte imbalance, or sepsis. Late diarrhea should be treated promptly with loperamide (see PRECAUTIONS, Information for Patients, for dosing recommendations for loperamide). Patients with diarrhea should be carefully monitored, should be given fluid and electrolyte replacement if they become dehydrated, and should be given antibiotic support if they develop ileus, fever, or severe neutropenia. After the first treatment, subsequent weekly chemotherapy treatments should be delayed in patients until return of pretreatment bowel function for at least 24 hours without need for antidiarrhea medication. If grade 2, 3, or 4 late diarrhea occurs subsequent doses of CAMPTOSAR should be decreased within the current cycle (see DOSAGE AND ADMINISTRATION).

Neutropenia

Deaths due to sepsis following severe neutropenia have been reported in patients treated with CAMPTOSAR. Neutropenic complications should be managed promptly with antibiotic support (see PRECAUTIONS). Therapy with CAMPTOSAR should be temporarily omitted during a cycle of therapy if neutropenic fever occurs or if the absolute neutrophil count drops $<1500/mm^3$. After the patient recovers to an absolute neutrophil count $\geq 1500/mm^3$, subsequent doses of CAMPTOSAR should be reduced depending upon the level of neutropenia observed (see DOSAGE AND ADMINISTRATION).

Routine administration of a colony-stimulating factor (CSF) is not necessary, but physicians may wish to consider CSF use in individual patients experiencing significant neutropenia.

Hypersensitivity

Hypersensitivity reactions including severe anaphylactic or anaphylactoid reactions have been observed.

Colitis/Ileus

Cases of colitis complicated by ulceration, bleeding, ileus, and infection have been observed. Patients experiencing ileus should receive prompt antibiotic support (see PRECAUTIONS).

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.

Thromboembolism

Thromboembolic events have been observed in patients receiving irinotecan-containing regimens; the specific cause of these events has not been determined.

Pregnancy

CAMPTOSAR may cause fetal harm when administered to a pregnant woman. Radioactivity related to ^{14}C-irinotecan crosses the placenta of rats following intravenous administration of 10 mg/kg (which in separate studies produced an irinotecan C_{max} and AUC about 3 and 0.5 times, respectively, the corresponding values in patients administered 125 mg/m^2). Administration of 6 mg/kg/day intravenous irinotecan to rats (which in separate studies produced an irinotecan C_{max} and AUC about 2 and 0.2 times, respectively, the corresponding values in patients administered 125 mg/m^2) and rabbits (about one-half the recommended human weekly starting dose on a mg/m^2 basis) during the period of organogenesis, is embryotoxic as characterized by increased post-implantation loss and decreased numbers of live fetuses. Irinotecan was teratogenic in rats at doses greater than 1.2 mg/kg/day (which in separate studies produced an irinotecan C_{max} and AUC about 2/3 and 1/40th, respectively, of the corresponding values in patients administered 125 mg/m^2) and in rabbits at 6.0 mg/kg/day (about one-half the recommended human weekly starting dose on a mg/m^2 basis). Teratogenic effects included a variety of external, visceral, and skeletal abnormalities. Irinotecan administered to rat dams for the period following organogenesis through weaning at doses of 6 mg/kg/day caused decreased learning ability and decreased female body weights in the offspring. There are no adequate and well-controlled studies of irinotecan in pregnant women. If the drug is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant while receiving treatment with CAMPTOSAR.

PRECAUTIONS.

General

Care of Intravenous Site: CAMPTOSAR Injection is administered by intravenous infusion. Care should be taken to avoid extravasation, and the infusion site should be monitored for signs of inflammation. Should extravasation occur, flushing the site with sterile water and applications of ice are recommended.

Premedication with Antiemetics: Irinotecan is emetigenic. It is recommended that patients receive premedication with antiemetic agents. In clinical studies of the weekly dosage schedule, the majority of patients received 10 mg of dexamethasone given in conjunction with another type of antiemetic agent, such as a 5-HT^3 blocker (e.g., ondansetron or granisetron). Antiemetic agents should be given on the day of treatment, starting at least 30 minutes before administration of CAMPTOSAR. Physicians should also consider providing patients with an antiemetic regimen (e.g., prochlorperazine) for subsequent use as needed.

Treatment of Cholinergic Symptoms: Prophylactic or therapeutic administration of 0.25 to 1 mg of intravenous or subcutaneous atropine should be considered (unless clinically contraindicated) in patients experiencing rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, abdominal cramping, or diarrhea (occurring during or shortly after infusion of CAMPTOSAR). These symptoms are expected to occur more frequently with higher irinotecan doses.

Patients at Particular Risk: In patients receiving either irinotecan/5-FU/LV or 5-FU/LV in the clinical trials, higher rates of hospitalization, neutropenic fever, thromboembolism, first-cycle treatment discontinuation, and early deaths were observed in patients with a baseline performance status of 2 than in patients with a baseline performance status of 0 or 1. Patients who had previously received pelvic/abdominal radiation and elderly patients with comorbid conditions should be closely monitored.

The use of CAMPTOSAR in patients with significant hepatic dysfunction has not been established. In clinical trials of either dosing schedule, irinotecan was not administered to patients with serum bilirubin >2.0 mg/dL, or transaminase >3 times the upper limit of normal if no liver metastasis, or transaminase >5 times the upper limit of normal with liver metastasis. However in clinical trials of the weekly dosage schedule, it has been noted that patients with modestly elevated baseline serum total bilirubin levels (1.0 to 2.0 mg/dL) have had a significantly greater likelihood of experiencing first-cycle grade 3 or 4 neutropenia than those with bilirubin levels that were less than 1.0 mg/dL (50.0% [19/38] versus 17.7% [47/226]; $p<0.001$). Patients with abnormal glucuronidation of bilirubin, such as those with Gilbert's syndrome, may also be at greater risk of myelosuppression when receiving therapy with CAMPTOSAR. An association between baseline bilirubin elevations and an increased risk of late diarrhea has not been observed in studies of the weekly dosage schedule.

Information for Patients

Patients and patients' caregivers should be informed of the expected toxic effects of CAMPTOSAR, particularly of its gastrointestinal complications, such as nausea, vomiting, abdominal cramping, diarrhea, and infection. Each patient should be instructed to have loperamide readily available and to begin treatment for late diarrhea (generally occurring more than 24 hours after administration of CAMPTOSAR) at the first episode of poorly formed or loose stools or the earliest onset of bowel movements more frequent than normally expected for the patient. One dosage regimen for loperamide used in clinical trials consisted of the following (Note: This dosage regimen exceeds the usual dosage recommendations for loperamide.): 4 mg at the first onset of late diarrhea and then 2 mg every 2 hours until the patient is diarrhea-free for at least 12 hours. During the night, the patient may take 4 mg of loperamide every 4 hours. Premedication with loperamide is not recommended. The use of drugs with laxative properties should be avoided because of the potential for exacerbation of diarrhea. Patients should be advised to contact their physician to discuss any laxative use.

Patients should be instructed to contact their physician or nurse if any of the following occur: diarrhea for the first time during treatment; black or bloody stools; symptoms of dehydration such as lightheadedness, dizziness, or faintness; inability to take fluids by mouth due to nausea or vomiting; inability to get diarrhea under control within 24 hours; or fever or evidence of infection.

Patients should be alerted to the possibility of alopecia.

Laboratory Tests

Careful monitoring of the white blood cell count with differential, hemoglobin, and platelet count is recommended before each dose of CAMPTOSAR.

Drug Interactions

The adverse effects of CAMPTOSAR, such as myelosuppression and diarrhea, would be expected to be exacerbated by other antineoplastic agents having similar adverse effects.

Patients who have previously received pelvic/abdominal irradiation are at increased risk of severe myelosuppression following the administration of CAMPTOSAR. The concurrent administration of CAMPTOSAR with irradiation has not been adequately studied and is not recommended.

Lymphocytopenia has been reported in patients receiving CAMPTOSAR, and it is possible that the administration of dexamethasone as antiemetic prophylaxis may have enhanced the likelihood of this effect. However, serious opportunistic infections have not been observed, and no complications have specifically been attributed to lymphocytopenia.

Hyperglycemia has also been reported in patients receiving CAMPTOSAR. Usually, this has been observed in patients with a history of diabetes mellitus or evidence of glucose intolerance prior to administration of CAMPTOSAR. It is probable that dexamethasone, given as antiemetic prophylaxis, contributed to hyperglycemia in some patients.

The incidence of akathisia in clinical trials of the weekly dosage schedule was greater (8.5%, 4/47 patients) when prochlorperazine was administered on the same day as CAMPTOSAR than when these drugs were given on separate days (1.3%, 1/80 patients). The 8.5% incidence of akathisia, however, is within the range reported for use of prochlorperazine when given as a premedication for other chemotherapies.

It would be expected that laxative use during therapy with CAMPTOSAR would worsen the incidence or severity of diarrhea, but this has not been studied.

In view of the potential risk of dehydration secondary to vomiting and/or diarrhea induced by CAMPTOSAR, the physician may wish to withhold diuretics during dosing with CAMPTOSAR and, certainly, during periods of active vomiting or diarrhea.

Drug-Laboratory Test Interactions

There are no known interactions between CAMPTOSAR and laboratory tests.

Carcinogenesis, Mutagenesis & Impairment of Fertility

Long-term carcinogenicity studies with irinotecan were not conducted. Rats were, however, administered intravenous doses of 2 mg/kg or 25 mg/kg irinotecan once per week for 13 weeks (in separate studies, the 25 mg/kg dose produced an irinotecan C_{max} and AUC that were about 7.0 times and 1.3 times the respective values in patients administered 125 mg/m^2 weekly) and were then allowed to recover for 91 weeks. Under these conditions, there was a significant linear trend with dose for the incidence of combined uterine horn endometrial stromal polyps and endometrial stromal sarcomas. Neither irinotecan nor SN-38 was mutagenic in the in vitro Ames assay. Irinotecan was clastogenic both in vitro (chromosome aberrations in Chinese hamster ovary cells) and in vivo (micronucleus test in mice). No significant adverse effects on fertility and general reproductive performance were

observed after intravenous administration of irinotecan in doses of up to 6 mg/kg/day to rats and rabbits. However, atrophy of male reproductive organs was observed after multiple daily irinotecan doses both in rodents at 20 mg/kg (which in separate studies produced an irinotecan C_{max} and AUC about 5 and 1 times, respectively, the corresponding values in patients administered 125 mg/m^2 weekly) and dogs at 0.4 mg/kg (which in separate studies produced an irinotecan C_{max} and AUC about one-half and 1/15th, respectively, the corresponding values in patients administered 125 mg/m^2 weekly).

Pregnancy

Pregnancy Category D—see WARNINGS.

Nursing Mothers

Radioactivity appeared in rat milk within 5 minutes of intravenous administration of radiolabeled irinotecan and was concentrated up to 65-fold at 4 hours after administration relative to plasma concentrations. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants, it is recommended that nursing be discontinued when receiving therapy with CAMPTOSAR.

Pediatric Use

The safety and effectiveness of CAMPTOSAR in pediatric patients have not been established.

Geriatric Use

Patients greater than 65 years of age should be closely monitored because of a greater risk of late diarrhea in this population (see CLINICAL PHARMACOLOGY, Pharmacokinetics in Special Populations and ADVERSE REACTIONS, Overview of Adverse Events). The starting dose of CAMPTOSAR in patients 70 years and older for the once-every-3-week-dosage schedule should be 300 mg/m^2 (see DOSAGE AND ADMINISTRATION).

ADVERSE REACTIONS

First-Line Combination Therapy

A total of 955 patients with metastatic colorectal cancer received the recommended regimens of irinotecan in combination with 5-FU/LV, 5-FU/LV alone, or irinotecan alone. In the two phase 3 studies, 370 patients received irinotecan in combination with 5-FU/LV, 362 patients received 5-FU/LV alone, and 223 patients received irinotecan alone. (See Table 10 in DOSAGE AND ADMINISTRATION for recommended combination-agent regimens.)

In Study 1, 49 (7.3%) patients died within 30 days of last study treatment: 21 (9.3%) received irinotecan in combination with 5-FU/LV, 15 (6.8%) received 5-FU/LV alone, and 13 (5.8%) received irinotecan alone. Deaths potentially related to treatment occurred in 2 (0.9%) patients who received irinotecan in combination with 5-FU/LV (2 neutropenic fever/sepsis), 3 (1.4%) patients who received 5-FU/LV alone (1 neutropenic fever/sepsis, 1 CNS bleeding during thrombocytopenia, 1 unknown) and 2 (0.9%) patients who received irinotecan alone (2 neutropenic fever). Deaths from any cause within 60 days of first study treatment were reported for 15 (6.7%) patients who received irinotecan in combination with

5-FU/LV, 16 (7.3%) patients who received 5-FU/LV alone, and 15 (6.7%) patients who received irinotecan alone. Discontinuations due to adverse events were reported for 17 (7.6%) patients who received irinotecan in combination with 5-FU/LV, 14 (6.4%) patients who received 5-FU/LV alone, and 26 (11.7%) patients who received irinotecan alone.

In Study 2, 10 (3.5%) patients died within 30 days of last study treatment: 6 (4.1%) received irinotecan in combination with 5-FU/LV and 4 (2.8%) received 5-FU/LV alone. There was one potentially treatment-death, which occurred in a patient who received irinotecan in combination with 5-FU/LV (0.7%, neutropenic sepsis). Deaths from any cause within 60 days of first study treatment were reported for 3 (2.1%) patients who received irinotecan in combination with 5-FU/LV and 2 (1.4%) patients who received 5-FU/LV alone. Discontinuations due to adverse events were reported for 9 (6.2%) patients who received irinotecan in combination with 5-FU/LV and 1 (0.7%) patient who received 5-FU/LV alone.

The most clinically significant adverse events for patients receiving irinotecan-based therapy were diarrhea, nausea, vomiting, neutropenia, and alopecia. The most clinically significant adverse events for patients receiving 5-FU/LV therapy were diarrhea, neutropenia, neutropenic fever, and mucositis. In Study 1, grade 4 neutropenia, neutropenic fever (defined as grade 2 fever and grade 4 neutropenia), and mucositis were observed less often with weekly irinotecan/5-FU/LV than with monthly administration of 5-FU/LV.

Tables 6 and 7 list the clinically relevant adverse events reported in Studies 1 and 2, respectively.

Table 6. Study 1: Percent (%) of Patients Experiencing Clinically Relevant Adverse Events in Combination Therapies[a]

Adverse Event	Study 1					
	Irinotecan + Bolus 5-FU/LV weekly x 4 q 6 weeks N=225		Bolus 5-FU/LV daily x 5 q 4 weeks N=219		Irinotecan weekly x 4 q 6 weeks N=223	
	Grade 1-4	Grade 3&4	Grade 1-4	Grade 3&4	Grade 1-4	Grade 3&4
TOTAL Adverse Events	100	53.3	100	45.7	99.6	45.7
GASTROINTESTINAL						
Diarrhea						
late	84.9	22.7	69.4	13.2	83.0	31.0
grade 3	--	15.1	--	5.9	--	18.4
grade 4	--	7.6	--	7.3	--	12.6
early	45.8	4.9	31.5	1.4	43.0	6.7
Nausea	79.1	15.6	67.6	8.2	81.6	16.1
Abdominal pain	63.1	14.6	50.2	11.5	67.7	13.0
Vomiting	60.4	9.7	46.1	4.1	62.8	12.1
Anorexia	34.2	5.8	42.0	3.7	43.9	7.2
Constipation	41.3	3.1	31.5	1.8	32.3	0.4
Mucositis	32.4	2.2	76.3	16.9	29.6	2.2
HEMATOLOGIC						
Neutropenia	96.9	53.8	98.6	66.7	96.4	31.4
grade 3	--	29.8	--	23.7	--	19.3
grade 4	--	24.0	--	42.5	--	12.1
Leukopenia	96.9	37.8	98.6	23.3	96.4	21.5
Anemia	96.9	8.4	98.6	5.5	96.9	4.5
Neutropenic fever	--	7.1	--	14.6	--	5.8
Thrombocytopenia	96.0	2.6	98.6	2.7	96.0	1.7
Neutropenic infection	--	1.8	--	0	--	2.2
BODY AS A WHOLE						
Asthenia	70.2	19.5	64.4	11.9	69.1	13.9
Pain	30.7	3.1	26.9	3.6	22.9	2.2
Fever	42.2	1.7	32.4	3.6	43.5	0.4
Infection	22.2	0	16.0	1.4	13.9	0.4
METABOLIC & NUTRITIONAL						
↑ Bilirubin	87.6	7.1	92.2	8.2	83.9	7.2
DERMATOLOGIC						
Exfoliative dermatitis	0.9	0	3.2	0.5	0	0
Rash	19.1	0	26.5	0.9	14.3	0.4
Alopecia[b]	43.1	--	26.5	--	46.1	--
RESPIRATORY						
Dyspnea	27.6	6.3	16.0	0.5	22.0	2.2
Cough	26.7	1.3	18.3	0	20.2	0.4
Pneumonia	6.2	2.7	1.4	1.0	3.6	1.3
NEUROLOGIC						
Dizziness	23.1	1.3	16.4	0	21.1	1.8
Somnolence	12.4	1.8	4.6	1.8	9.4	1.3
Confusion	7.1	1.8	4.1	0	2.7	0
CARDIOVASCULAR						
Vasodilatation	9.3	0.9	5.0	0	9.0	0
Hypotension	5.8	1.3	2.3	0.5	5.8	1.7
Thromboembolic events[c]	9.3	--	11.4	--	5.4	--

[a]Severity of adverse events based on NCI CTC (version 1.0)

[b]Complete hair loss = Grade 2

[c]Includes angina pectoris, arterial thrombosis, cerebral infarct, cerebrovascular accident, deep thrombophlebitis, embolus lower extremity, heart arrest, myocardial infact, myocardial ischemia, peripheral vascular disorder, pulmonary embolus, sudden death, thrombophlebitis, thrombosis, vascular disorder.

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Table 7. Study 2: Percent (%) of Patients Experiencing Clinically Relevant Adverse Events in Combination Therapies[a]

Adverse Event	Study 2			
	Irinotecan + 5-FU/LV infusional d 1&2 q 2 weeks N= 145		5-FU/LV infusional d 1&2 q 2 weeks N=143	
	Grade 1-4	Grade 3&4	Grade 1-4	Grade 3&4
TOTAL Adverse Events	100	72.4	100	39.2
GASTROINTESTINAL				
Diarrhea				
late	72.4	14.4	44.8	6.3
grade 3	--	10.3	--	4.2
grade 4	--	4.1	--	2.1
Cholinergic syndrome[b]	28.3	1.4	0.7	0
Nausea	66.9	2.1	55.2	3.5
Abdominal pain	17.2	2.1	16.8	0.7
Vomiting	44.8	3.5	32.2	2.8
Anorexia	35.2	2.1	18.9	0.7
Constipation	30.3	0.7	25.2	1.4
Mucositis	40.0	4.1	28.7	2.8
HEMATOLOGIC				
Neutropenia	82.5	46.2	47.9	13.4
grade 3	--	36.4	--	12.7
grade 4	--	9.8	--	0.7
Leukopenia	81.3	17.4	42.0	3.5
Anemia	97.2	2.1	90.9	2.1
Neutropenic fever	--	3.4	--	0.7
Thrombocytopenia	32.6	0	32.2	0
Neutropenic infection	--	2.1	--	0
BODY AS A WHOLE				
Asthenia	57.9	9.0	48.3	4.2
Pain	64.1	9.7	61.5	8.4
Fever	22.1	0.7	25.9	0.7
Infection	35.9	7.6	33.6	3.5
METABOLIC & NUTRITIONAL				
↑ Bilirubin	19.1	3.5	35.9	10.6
DERMATOLOGIC				
Hand & foot syndrome	10.3	0.7	12.6	0.7
Cutaneous signs	17.2	0.7	20.3	0
Alopecia[c]	56.6	--	16.8	--
RESPIRATORY				
Dyspnea	9.7	1.4	4.9	0
CARDIOVASCULAR				
Hypotension	3.4	1.4	0.7	0
Thromboembolic events[d]	11.7	--	5.6	--

[a] Severity of adverse events based on NCI CTC (version 1.0)

[b] Includes rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, abdominal cramping or diarrhea (occurring during or shortly after infusion of irinotecan)

[c] Complete hair loss = Grade 2

[d] Includes angina pectoris, arterial thrombosis, cerebral infarct, cerebrovascular accident, deep thrombophlebitis, embolus lower extremity, heart arrest, myocardial infact, myocardial ischemia, peripheral vascular disorder, pulmonary embolus, sudden death, thrombophlebitis, thrombosis, vascular disorder.

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Second-Line Single-Agent Therapy

Weekly Dosage Schedule

In three clinical studies evaluating the weekly dosage schedule, 304 patients with metastatic carcinoma of the colon or rectum that had recurred or progressed following 5-FU-based therapy were treated with CAMPTOSAR. Seventeen of the patients died within 30 days of the administration of CAMPTOSAR; in five cases (1.6%, 5/304), the deaths were potentially drug-related. These five patients experienced a constellation of medical events that included known effects of CAMPTOSAR. One of these patients died of neutropenic sepsis without fever. Neutropenic fever occurred in nine (3.0%) other patients; these patients recovered with supportive care.

One hundred nineteen (39.1%) of the 304 patients were hospitalized a total of 156 times because of adverse events; 81 (26.6%) patients were hospitalized for events judged to be related to administration of CAMPTOSAR. The primary reasons for drug-related hospitalization were diarrhea, with or without nausea and/or vomiting (18.4%); neutropenia/leukopenia, with or without diarrhea and/or fever (8.2%); and nausea and/or vomiting (4.9%).

Adjustments in the dose of CAMPTOSAR were made during the cycle of treatment and for subsequent cycles based on individual patient tolerance. The first dose of at least one cycle of CAMPTOSAR was reduced for 67% of patients who began the studies at the 125-mg/m^2 starting dose. Within-cycle dose reductions were required for 32% of the cycles initiated at the 125-mg/m^2 dose level. The most common reasons for dose reduction were late diarrhea, neutropenia, and leukopenia. Thirteen (4.3%) patients discontinued treatment with CAMPTOSAR because of adverse events. The adverse events in Table 8 are based on the experience of the 304 patients enrolled in the three studies described in the CLINICAL STUDIES, Studies Evaluating the Weekly Dosage Schedule, section.

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Table 8. Adverse Events Occurring in >10% of 304 Previously Treated Patients with Metastatic Carcinoma of the Colon or Rectum[a]

Body System & Event	% of Patients Reporting	
	NCI Grades 1-4	NCI Grades 3 & 4
GASTROINTESTINAL		
Diarrhea (late)[b]	88	31
7-9 stools/day (grade 3)	----	(16)
≥10 stools/day (grade 4)	----	(14)
Nausea	86	17
Vomiting	67	12
Anorexia	55	6
Diarrhea (early)[c]	51	8
Constipation	30	2
Flatulence	12	0
Stomatitis	12	1
Dyspepsia	10	0
HEMATOLOGIC		
Leukopenia	63	28
Anemia	60	7
Neutropenia	54	26
500 to <1000/mm^3 (grade 3)	----	(15)
<500/mm^3 (grade 4)	----	(12)
BODY AS A WHOLE		
Asthenia	76	12
Abdominal cramping/pain	57	16
Fever	45	1
Pain	24	2
Headache	17	1
Back pain	14	2
Chills	14	0
Minor infection[d]	14	0
Edema	10	1
Abdominal enlargement	10	0
METABOLIC & NUTRITIONAL		
↓ Body weight	30	1
Dehydration	15	4
↑ Alkaline phosphatase	13	4
↑ SGOT	10	1
DERMATOLOGIC		
Alopecia	60	NA[e]
Sweating	16	0
Rash	13	1
RESPIRATORY		
Dyspnea	22	4
↑ Coughing	17	0
Rhinitis	16	0
NEUROLOGIC		
Insomnia	19	0
Dizziness	15	0
CARDIOVASCULAR		
Vasodilation (Flushing)	11	0

[a] Severity of adverse events based on NCI CTC (version 1.0)
[b] Occurring > 24 hours after administration of CAMPTOSAR
[c] Occurring ≤24 hours after administration of CAMPTOSAR
[d] Primarily upper respiratory infections
[e] Not applicable; complete hair loss = NCI grade 2

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601 ***Once-Every-3-Week Dosage Schedule***

602 A total of 535 patients with metastatic colorectal cancer whose disease had recurred or
603 progressed following prior 5-FU therapy participated in the two phase 3 studies: 316 received

irinotecan, 129 received 5-FU, and 90 received best supportive care. Eleven (3.5%) patients treated with irinotecan died within 30 days of treatment. In three cases (1%, 3/316), the deaths were potentially related to irinotecan treatment and were attributed to neutropenic infection, grade 4 diarrhea, and asthenia, respectively. One (0.8%, 1/129) patient treated with 5-FU died within 30 days of treatment; this death was attributed to grade 4 diarrhea.

Hospitalizations due to serious adverse events (whether or not related to study treatment) occurred at least once in 60% (188/316) of patients who received irinotecan, 63% (57/90) who received best supportive care, and 39% (50/129) who received 5-FU-based therapy. Eight percent of patients treated with irinotecan and 7% treated with 5-FU-based therapy discontinued treatment due to adverse events.

Of the 316 patients treated with irinotecan, the most clinically significant adverse events (all grades, 1-4) were diarrhea (84%), alopecia (72%), nausea (70%), vomiting (62%), cholinergic symptoms (47%), and neutropenia (30%). Table 9 lists the grade 3 and 4 adverse events reported in the patients enrolled to all treatment arms of the two studies described in the CLINICAL STUDIES, Studies Evaluating the Once-Every-3-Week Dosage Schedule, section.

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Table 9. Percent Of Patients Experiencing Grade 3 & 4 Adverse Events In Comparative Studies Of Once-Every-3-Week Irinotecan Therapy[a]

	Study 1		Study 2	
Adverse Event	**Irinotecan N=189**	**BSC [b] N=90**	**Irinotecan N=127**	5-FU N=129
TOTAL Grade ¾ Adverse Events	79	67	69	54
GASTROINTESTINAL				
Diarrhea	22	6	22	11
Vomiting	14	8	14	5
Nausea	14	3	11	4
Abdominal pain	14	16	9	8
Constipation	10	8	8	6
Anorexia	5	7	6	4
Mucositis	2	1	2	5
HEMATOLOGIC				
Leukopenia/Neutropenia	22	0	14	2
Anemia	7	6	6	3
Hemorrhage	5	3	1	3
Thrombocytopenia	1	0	4	2
Infection				
without grade 3/4 neutropenia	8	3	1	4
with grade 3/4 neutropenia	1	0	2	0
Fever				
without grade 3/4 neutropenia	2	1	2	0
with grade 3/4 neutropenia	2	0	4	2
BODY AS A WHOLE				
Pain	19	22	17	13
Asthenia	15	19	13	12
METABOLIC & NUTRITIONAL				
Hepatic [c]	9	7	9	6
DERMATOLOGIC				
Hand & foot syndrome	0	0	0	5
Cutaneous signs [d]	2	0	1	3
RESPIRATORY [e]	10	8	5	7
NEUROLOGIC [f]	12	13	9	4
CARDIOVASCULAR [g]	9	3	4	2
OTHER [h]	32	28	12	14

[a] Severity of adverse events based on NCI CTC (version 1.0)
[b] BSC = best supportive care
[c] Hepatic includes events such as ascites and jaundice
[d] Cutaneous signs include events such as rash
[e] Respiratory includes events such as dyspnea and cough
[f] Neurologic includes events such as somnolence
[g] Cardiovascular includes events such as dysrhythmias, ischemia, and mechanical cardiac dysfunction
[h] Other includes events such as accidental injury, hepatomegaly, syncope, vertigo, and weight loss

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623 **Overview of Adverse Events**

624 *Gastrointestinal:* Nausea, vomiting, and diarrhea are common adverse events following
625 treatment with CAMPTOSAR and can be severe. When observed, nausea and vomiting
626 usually occur during or shortly after infusion of CAMPTOSAR. In the clinical studies testing
627 the every 3-week-dosage schedule, the median time to the onset of late diarrhea was 5 days
628 after irinotecan infusion. In the clinical studies evaluating the weekly dosage schedule, the

median time to onset of late diarrhea was 11 days following administration of CAMPTOSAR. For patients starting treatment at the 125-mg/m^2 weekly dose, the median duration of any grade of late diarrhea was 3 days. Among those patients treated at the 125-mg/m^2 weekly dose who experienced grade 3 or 4 late diarrhea, the median duration of the entire episode of diarrhea was 7 days. The frequency of grade 3 or 4 late diarrhea was somewhat greater in patients starting treatment at 125 mg/m^2 than in patients given a 100-mg/m^2 weekly starting dose (34% [65/193] versus 23% [24/102]; p=0.08). The frequency of grade 3 and 4 late diarrhea by age was significantly greater in patients ≥65 years than in patients <65 years (40% [53/133] versus 23% [40/171]; p = 0.002). In one study of the weekly dosage treatment, the frequency of grade 3 and 4 late diarrhea was significantly greater in male than in female patients (43% [25/58] versus 16% [5/32]; p = 0.01), but there were no gender differences in the frequency of grade 3 and 4 late diarrhea in the other two studies of the weekly dosage treatment schedule. Colonic ulceration, sometimes with gastrointestinal bleeding, has been observed in association with administration of CAMPTOSAR.

Hematology: CAMPTOSAR commonly causes neutropenia, leukopenia (including lymphocytopenia), and anemia. Serious thrombocytopenia is uncommon. When evaluated in the trials of weekly administration, the frequency of grade 3 and 4 neutropenia was significantly higher in patients who received previous pelvic/abdominal irradiation than in those who had not received such irradiation (48% [13/27] versus 24% [67/277]; p = 0.04). In these same studies, patients with baseline serum total bilirubin levels of 1.0 mg/dL or more also had a significantly greater likelihood of experiencing first-cycle grade 3 or 4 neutropenia than those with bilirubin levels that were less than 1.0 mg/dL (50% [19/38] versus 18% [47/266]; p<0.001). There were no significant differences in the frequency of grade 3 and 4 neutropenia by age or gender. In the clinical studies evaluating the weekly dosage schedule, neutropenic fever (concurrent NCI grade 4 neutropenia and fever of grade 2 or greater) occurred in 3% of the patients; 6% of patients received G-CSF for the treatment of neutropenia. NCI grade 3 or 4 anemia was noted in 7% of the patients receiving weekly treatment; blood transfusions were given to 10% of the patients in these trials.

Body as a Whole: Asthenia, fever, and abdominal pain are generally the most common events of this type.

Cholinergic Symptoms: Patients may have cholinergic symptoms of rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, and intestinal hyperperistalsis that can cause abdominal cramping and early diarrhea. If these symptoms occur, they manifest during or shortly after drug infusion. They are thought to be related to the anticholinesterase activity of the irinotecan parent compound and are expected to occur more frequently with higher irinotecan doses.

Hepatic: In the clinical studies evaluating the weekly dosage schedule, NCI grade 3 or 4 liver enzyme abnormalities were observed in fewer than 10% of patients. These events typically occur in patients with known hepatic metastases.

Dermatologic: Alopecia has been reported during treatment with CAMPTOSAR. Rashes have also been reported but did not result in discontinuation of treatment.

Respiratory: Severe pulmonary events are infrequent. In the clinical studies evaluating the weekly dosage schedule, NCI grade 3 or 4 dyspnea was reported in 4% of patients. Over half the patients with dyspnea had lung metastases; the extent to which malignant pulmonary

involvement or other preexisting lung disease may have contributed to dyspnea in these patients is unknown.

Neurologic: Insomnia and dizziness can occur, but are not usually considered to be directly related to the administration of CAMPTOSAR. Dizziness may sometimes represent symptomatic evidence of orthostatic hypotension in patients with dehydration.

Cardiovascular: Vasodilation (flushing) may occur during administration of CAMPTOSAR. Bradycardia may also occur, but has not required intervention. These effects have been attributed to the cholinergic syndrome sometimes observed during or shortly after infusion of CAMPTOSAR. Thromboembolic events have been observed in patients receiving CAMPTOSAR; the specific cause of these events has not been determined.

Other Non-U.S. Clinical Trials

Irinotecan has been studied in over 1100 patients in Japan. Patients in these studies had a variety of tumor types, including cancer of the colon or rectum, and were treated with several different doses and schedules. In general, the types of toxicities observed were similar to those seen in US trials with CAMPTOSAR. There is some information from Japanese trials that patients with considerable ascites or pleural effusions were at increased risk for neutropenia or diarrhea. A potentially life-threatening pulmonary syndrome, consisting of dyspnea, fever, and a reticulonodular pattern on chest x-ray, was observed in a small percentage of patients in early Japanese studies. The contribution of irinotecan to these preliminary events was difficult to assess because these patients also had lung tumors and some had preexisting nonmalignant pulmonary disease. As a result of these observations, however, clinical studies in the United States have enrolled few patients with compromised pulmonary function, significant ascites, or pleural effusions.

Post-Marketing Experience

The following events have been identified during post-marketing use of CAMPTOSAR in clinical practice. Cases of colitis complicated by ulceration, bleeding, ileus, or infection have been observed. There have been rare cases of renal impairment and acute renal failure, generally in patients who became infected and/or volume depleted from severe gastrointestinal toxicities (see WARNINGS). Hypersensitivity reactions including severe anaphylactic or anaphylactoid reactions have also been observed (see WARNINGS).

OVERDOSAGE

In U.S. phase 1 trials, single doses of up to 345 mg/m^2 of irinotecan were administered to patients with various cancers. Single doses of up to 750 mg/m^2 of irinotecan have been given in non-U.S. trials. The adverse events in these patients were similar to those reported with the recommended dosage and regimen. There is no known antidote for overdosage of CAMPTOSAR. Maximum supportive care should be instituted to prevent dehydration due to diarrhea and to treat any infectious complications.

DOSAGE AND ADMINISTRATION

Combination-Agent Dosage

Dosage Regimens

CAMPTOSAR Injection in Combination with 5-Fluorouracil (5-FU) and Leucovorin (LV)

CAMPTOSAR should be administered as an intravenous infusion over 90 minutes (see Preparation of Infusion Solution). For all regimens, the dose of LV should be administered immediately after CAMPTOSAR, with the administration of 5-FU to occur immediately after receipt of LV. CAMPTOSAR should be used as recommended; the currently recommended regimens are shown in Table 10.

Table 10. Combination-Agent Dosage Regimens & 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 IV over 90 min, d 1,8,15,22 20 mg/m^2 IV bolus, d 1,8,15,22 500 mg/m^2 IV bolus, d 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 IV over 90 min, d 1,15,29 200 mg/m^2 IV over 2 h, d 1,2,15,16,29,30 400 mg/m^2 IV bolus, d 1,2,15,16,29,30 600 mg/m^2 IV over 22 h, d 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 since such patients were not included in clinical studies. It is recommended that patients receive premedication with antiemetic agents. Prophylactic or therapeutic administration of atropine should be considered in patients experiencing cholinergic symptoms. See PRECAUTIONS, General.

Dose Modifications

Patients should be carefully monitored for toxicity and assessed prior to each treatment. Doses of CAMPTOSAR and 5-FU should be modified as necessary to accommodate individual patient tolerance to treatment. Based on the recommended dose-levels described in Table 10, Combination-Agent Dosage Regimens & Dose Modifications, subsequent doses should be adjusted as suggested in Table 11, Recommended Dose Modifications for Combination Schedules. All dose modifications should be based on the worst preceding toxicity. After the first treatment, patients with active diarrhea should return to pretreatment bowel function without requiring antidiarrhea medications for at least 24 hours before the next chemotherapy administration.

A new cycle of therapy should not begin until the toxicity has recovered to NCI grade 1 or less. Treatment may be delayed 1 to 2 weeks to allow for recovery from treatment-related toxicity. If the patient has not recovered, consideration should be given to discontinuing therapy. Provided intolerable toxicity does not develop, treatment with additional cycles of CAMPTOSAR/5-FU/LV may be continued indefinitely as long as patients continue to experience clinical benefit.

Table 11. Recommended Dose Modifications for CAMPTOSAR/5-Fluorouracil(5-FU)/Leucovorin (LV) Combination Schedules

Patients should return to pre-treatment bowel function without requiring antidiarrhea medications for at least 24 hours before the next chemotherapy administration. A new cycle of therapy should not begin until the granulocyte count has recovered to ≥1500/mm^3, and the platelet count has recovered to ≥100,000/mm^3, and treatment-related diarrhea is fully resolved. Treatment should be delayed 1 to 2 weeks to allow for recovery from treatment-related toxicities. If the patient has not recovered after a 2-week delay, consideration should be given to discontinuing therapy

Toxicity NCI CTC grade[a] (Value)	During a Cycle of Therapy	At the Start of Subsequent Cycles of Therapy[b]
No toxicity	Maintain dose level	Maintain dose level
Neutropenia		
1 (1500 to 1999/mm^3)	Maintain dose level	Maintain dose level
2 (1000 to 1499/mm^3)	↓ 1 dose level	Maintain dose level
3 (500 to 999/mm^3)	Omit dose until resolved to ≤ grade 2, then ↓ 1 dose level	↓ 1 dose level
4 (<500/mm^3)	Omit dose until resolved to ≤ grade 2, then ↓ 2 dose levels	↓ 2 dose levels
Neutropenic fever	Omit dose until resolved, then ↓ 2 dose levels	
Other hematologic toxicities	Dose modifications for leukopenia or thrombocytopenia during a cycle of therapy and at the start of subsequent cycles of therapy are also based on NCI toxicity criteria and are the same as recommended for neutropenia above.	
Diarrhea		
1 (2-3 stools/day > pretx[c])	Delay dose until resolved to baseline, then give same dose	Maintain dose level
2 (4-6 stools/day > pretx)	Omit dose until resolved to baseline, then ↓ 1 dose level	Maintain dose level
3 (7-9 stools/day > pretx)	Omit dose until resolved to baseline, then ↓ 1 dose level	↓ 1 dose level
4 (≥ 10 stools/day > pretx)	Omit dose until resolved to baseline, then ↓ 2 dose levels	↓ 2 dose levels
Other nonhematologic toxicities[d]		
1	Maintain dose level	Maintain dose level
2	Omit dose until resolved to ≤ grade 1, then ↓ 1 dose level	Maintain dose level
3	Omit dose until resolved to ≤ grade 2, then ↓ 1 dose level	↓ 1 dose level
4	Omit dose until resolved to ≤ grade 2, then ↓ 2 dose levels	↓ 2 dose levels
	For mucositis/stomatitis decrease only 5-FU, not CAMPTOSAR	*For mucositis/stomatitis decrease only 5-FU, not CAMPTOSAR.*

[a] National Cancer Institute Common Toxicity Criteria (version 1.0)
[b] Relative to the starting dose used in the previous cycle
[c] Pretreatment
[d] Excludes alopecia, anorexia, asthenia

Single-Agent Dosage Schedules

Dosage Regimens

CAMPTOSAR should be administered as an intravenous infusion over 90 minutes for both the weekly and once-every-3-week dosage schedules (see Preparation of Infusion Solution). Single-agent dosage regimens are shown in Table 12.

Table 12. Single-Agent Regimens of CAMPTOSAR and Dose Modifications

Weekly Regimen[a]	125 mg/m^2 IV over 90 min, d 1,8,15,22 then 2-wk rest		
	Starting Dose & Modified Dose Levels[c] **(mg/m^2)**		
	Starting Dose	Dose Level -1	Dose Level -2
	125	100	75
Once-Every-3-Week Regimen[b]	350 mg/m^2 IV over 90 min, once every 3 wks[c]		
	Starting Dose & Modified Dose Levels (mg/m^2)		
	Starting Dose	Dose Level -1	Dose Level -2
	350	300	250

[a]Subsequent doses may be adjusted as high as 150 mg/m^2 or to as low as 50 mg/m^2 in 25 to 50 mg/m^2 decrements depending upon individual patient tolerance.
[b]Subsequent doses may be adjusted as low as 200 mg/m^2 in 50 mg/m^2 decrements depending upon individual patient tolerance.
[c] Provided intolerable toxicity does not develop, treatment with additional cycles may be continued indefinitely as long as patients continue to experience clinical benefit.

A reduction in the starting dose by one dose level of CAMPTOSAR may be considered for patients with any of the following conditions: age ≥ 65 years, prior pelvic/abdominal radiotherapy, performance status of 2, or increased bilirubin levels. Dosing for patients with bilirubin >2 mg/dL cannot be recommended since such patients were not included in clinical studies.

It is recommended that patients receive premedication with antiemetic agents. Prophylactic or therapeutic administration of atropine should be considered in patients experiencing cholinergic symptoms. See PRECAUTIONS, General.

Dose Modifications

Patients should be carefully monitored for toxicity and doses of CAMPTOSAR should be modified as necessary to accommodate individual patient tolerance to treatment. Based on recommended dose-levels described in Table 12, Single-Agent Regimens of CAMPTOSAR and Dose Modifications, subsequent doses should be adjusted as suggested in Table 13, Recommended Dose Modifications for Single-Agent Schedules. All dose modifications should be based on the worst preceding toxicity.

A new cycle of therapy should not begin until the toxicity has recovered to NCI grade 1 or less. Treatment may be delayed 1 to 2 weeks to allow for recovery from treatment-related toxicity. If the patient has not recovered, consideration should be given to discontinuing this combination therapy. Provided intolerable toxicity does not develop, treatment with additional cycles of CAMPTOSAR may be continued indefinitely as long as patients continue to experience clinical benefit.

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Table 13. Recommended Dose Modifications For Single-Agent Schedules[a]

A new cycle of therapy should not begin until the granulocyte count has recovered to ≥1500/mm^3, and the platelet count has recovered to ≥100,000/mm^3, and treatment-related diarrhea is fully resolved. Treatment should be delayed 1 to 2 weeks to allow for recovery from treatment-related toxicities. If the patient has not recovered after a 2-week delay, consideration should be given to discontinuing CAMPTOSAR.

Worst Toxicity NCI Grade[b] (Value)	During a Cycle of Therapy	At the Start of the Next Cycles of Therapy (After Adequate Recovery), Compared with the Starting Dose in the Previous Cycle[a]	
	Weekly	Weekly	Once Every 3 Week
No toxicity	Maintain dose level	↑ 25 mg/m^2 up to a maximum dose of 150 mg/m^2	Maintain dose level
Neutropenia			
1 (1500 to 1999/mm^3)	Maintain dose level	Maintain dose level	Maintain dose level
2 (1000 to 1499/mm^3)	↓ 25 mg/m^2	Maintain dose level	Maintain dose level
3 (500 to 999/mm^3)	Omit dose until resolved to ≤ grade 2, then ↓ 25 mg/m^2	↓ 25 mg/m^2	↓ 50 mg/m^2
4 (<500/mm^3)	Omit dose until resolved to ≤ grade 2, then ↓ 50 mg/m^2	↓ 50 mg/m^2	↓ 50 mg/m^2
Neutropenic fever	Omit dose until resolved, then ↓ 50 mg/m^2 when resolved	↓ 50 mg/m^2	↓ 50 mg/m^2
Other hematologic toxicities	Dose modifications for leukopenia, thrombocytopenia, and anemia during a cycle of therapy and at the start of subsequent cycles of therapy are also based on NCI toxicity criteria and are the same as recommended for neutropenia above.		
Diarrhea			
1 (2-3 stools/day > pretx[c])	Maintain dose level	Maintain dose level	Maintain dose level
2 (4-6 stools/day > pretx)	↓ 25 mg/m^2	Maintain dose level	Maintain dose level
3 (7-9 stools/day > pretx)	Omit dose until resolved to ≤ grade 2, then ↓ 25 mg/m^2	↓ 25 mg/m^2	↓ 50 mg/m^2
4 (≥ 10 stools/day > pretx)	Omit dose until resolved to ≤ grade 2, then ↓ 50 mg/m^2	↓ 50 mg/m^2	↓ 50 mg/m^2
Other nonhematologic[d] toxicities			
1	Maintain dose level	Maintain dose level	Maintain dose level
2	↓ 25 mg/m^2	↓ 25 mg/m^2	↓ 50 mg/m^2
3-4	Omit dose until resolved to ≤ grade 2, then ↓ 25 mg/m^2	↓ 25 mg/m^2	↓ 50 mg/m^2
	Omit dose until resolved to ≤ grade 2, then ↓ 50 mg/m^2	↓ 50 mg/m^2	↓ 50 mg/m^2

[a] All dose modifications should be based on the worst preceding toxicity

[b] National Cancer Institute Common Toxicity Criteria (version 1.0)

[c] Pretreatment

[d] Excludes alopecia, anorexia, asthenia

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Preparation & Administration Precautions

As with other potentially toxic anticancer agents, care should be exercised in the handling and preparation of infusion solutions prepared from CAMPTOSAR Injection. The use of gloves is recommended. If a solution of CAMPTOSAR contacts the skin, wash the skin immediately and thoroughly with soap and water. If CAMPTOSAR contacts the mucous membranes, flush thoroughly with water. Several published guidelines for handling and disposal of anticancer agents are available.[1-7]

Preparation of Infusion Solution

Inspect vial contents for particulate matter and repeat inspection when drug product is withdrawn from vial into syringe.

CAMPTOSAR Injection must be diluted prior to infusion. CAMPTOSAR should be diluted in 5% Dextrose Injection, USP, (preferred) or 0.9% Sodium Chloride Injection, USP, to a final concentration range of 0.12 to 2.8 mg/mL. In most clinical trials, CAMPTOSAR was administered in 250 mL to 500 mL of 5% Dextrose Injection, USP.

The solution is physically and chemically stable for up to 24 hours at room temperature (approximately 25°C) and in ambient fluorescent lighting. Solutions diluted in 5% Dextrose Injection, USP, and stored at refrigerated temperatures (approximately 2° to 8°C), and protected from light are physically and chemically stable for 48 hours. Refrigeration of admixtures using 0.9% Sodium Chloride Injection, USP, is not recommended due to a low and sporadic incidence of visible particulates. Freezing CAMPTOSAR and admixtures of CAMPTOSAR may result in precipitation of the drug and should be avoided. Because of possible microbial contamination during dilution, it is advisable to use the admixture prepared with 5% Dextrose Injection, USP, within 24 hours if refrigerated (2° to 8°C, 36° to 46°F). In the case of admixtures prepared with 5% Dextrose Injection, USP, or Sodium Chloride Injection, USP, the solutions should be used within 6 hours if kept at room temperature (15° to 30°C, 59° to 86°F).

Other drugs should not be added to the infusion solution. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

HOW SUPPLIED

Each mL of CAMPTOSAR Injection contains 20 mg irinotecan (on the basis of the trihydrate salt); 45 mg sorbitol; and 0.9 mg lactic acid. When necessary, pH has been adjusted to 3.5 (range, 3.0 to 3.8) with sodium hydroxide or hydrochloric acid.

CAMPTOSAR Injection is available in single-dose amber glass vials in the following package sizes:

2 mL NDC 0009-7529-02

5 mL NDC 0009-7529-01

This is packaged in a backing/plastic blister to protect against inadvertent breakage and leakage. The vial should be inspected for damage and visible signs of leaks before removing the backing/plastic blister. If damaged, incinerate the unopened package.

Store at controlled room temperature 15° to 30°C (59° to 86°F). Protect from light. It is recommended that the vial (and backing/plastic blister) should remain in the carton until the time of use.

Rx only

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4. Clinical Oncological Society of Australia. Guidelines and recommendations for safe handling of antineoplastic agents. Med J Australia 1983;1:426-8.
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Richard Pazdur
5/16/02 12:47:42 PM

Camptosar®
irinotecan hydrochloride injection

For Intravenous Use Only

WARNINGS

CAMPTOSAR Injection should be administered only under the supervision of a physician who is experienced in the use of cancer chemotherapeutic agents. Appropriate management of complications is possible only when adequate diagnostic and treatment facilities are readily available. CAMPTOSAR can induce both early and late forms of diarrhea that appear to be mediated by different mechanisms. Both forms of diarrhea may be severe. Early diarrhea (occurring during or shortly after infusion of CAMPTOSAR) may be accompanied by cholinergic symptoms of rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, and intestinal hyperperistalsis that can cause abdominal cramping. Early diarrhea and other cholinergic symptoms may be prevented or ameliorated by atropine (see PRECAUTIONS, General). Late diarrhea (generally occurring more than 24 hours after administration of CAMPTOSAR) can be life threatening since it may be prolonged and may lead to dehydration, electrolyte imbalance, or sepsis. Late diarrhea should be treated promptly with loperamide. Patients with diarrhea should be carefully monitored and given fluid and electrolyte replacement if they become dehydrated or antibiotic therapy if they develop ileus, fever, or severe neutropenia (see WARNINGS). Administration of CAMPTOSAR should be interrupted and subsequent doses reduced if severe diarrhea occurs (see DOSAGE AND ADMINISTRATION).

Severe myelosuppression may occur (see WARNINGS).

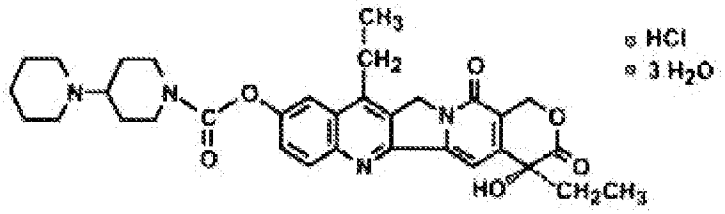
DESCRIPTION

CAMPTOSAR Injection (irinotecan hydrochloride injection) is an antineoplastic agent of the topoisomerase I inhibitor class. Irinotecan hydrochloride was clinically investigated as CPT-11.

CAMPTOSAR is supplied as a sterile, pale yellow, clear, aqueous solution. It is available in two single-dose sizes: 2 mL-fill vials contain 40 mg irinotecan hydrochloride and 5 mL-fill vials contain 100 mg irinotecan hydrochloride. Each milliliter of solution contains 20 mg of irinotecan hydrochloride (on the basis of the trihydrate salt), 45 mg of sorbitol NF powder, and 0.9 mg of lactic acid, USP. The pH of the solution has been adjusted to 3.5 (range, 3.0 to 3.8) with sodium hydroxide or hydrochloric acid. CAMPTOSAR is intended for dilution with 5% Dextrose Injection, USP (D5W), or 0.9% Sodium Chloride Injection, USP, prior to intravenous infusion. The preferred diluent is 5% Dextrose Injection, USP.

Irinotecan hydrochloride is a semisynthetic derivative of camptothecin, an alkaloid extract from plants such as *Camptotheca acuminata* or is chemically synthesized.

The chemical name is *(**S**)*-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1*H*-pyrano[3',4':6,7]-indolizino[1,2-b]quinolin-9-yl-[1,4'bipiperidine]-1'-carboxylate, monohydrochloride, trihydrate. Its structural formula is as follows:



Irinotecan Hydrochloride

Irinotecan hydrochloride is a pale yellow to yellow crystalline powder, with the empirical formula $C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3H_2O$ and a molecular weight of 677.19. It is slightly soluble in water and organic solvents.

CLINICAL PHARMACOLOGY

Irinotecan is a derivative of camptothecin. Camptothecins interact specifically with the enzyme topoisomerase I which relieves torsional strain in DNA by inducing reversible single-strand breaks. Irinotecan and its active metabolite SN-38 bind to the topoisomerase I-DNA complex and prevent religation of these single-strand breaks. Current research suggests that the cytotoxicity of irinotecan is due to double-strand DNA damage produced during DNA synthesis when replication enzymes interact with the ternary complex formed by topoisomerase I, DNA, and either irinotecan or SN-38. Mammalian cells cannot efficiently repair these double-strand breaks.

Irinotecan serves as a water-soluble precursor of the lipophilic metabolite SN-38. SN-38 is formed from irinotecan by carboxylesterase-mediated cleavage of the carbamate bond between the camptothecin moiety and the dipiperidino side chain. SN-38 is approximately 1000 times as potent as irinotecan as an inhibitor of topoisomerase I purified from human and rodent tumor cell lines. In vitro cytotoxicity assays show that the potency of SN-38 relative to irinotecan varies from 2- to 2000-fold. However, the plasma area under the concentration versus time curve (AUC) values for SN-38 are 2% to 8% of irinotecan and SN-38 is 95% bound to plasma proteins compared to approximately 50% bound to plasma proteins for irinotecan (see Pharmacokinetics). The precise contribution of SN-38 to the activity of CAMPTOSAR is thus unknown. Both irinotecan and SN-38 exist in an active lactone form and an inactive hydroxy acid anion form. A pH-dependent equilibrium exists between the two forms such that an acid pH promotes the formation of the lactone, while a more basic pH favors the hydroxy acid anion form.

Administration of irinotecan has resulted in antitumor activity in mice bearing cancers of rodent origin and in human carcinoma xenografts of various histological types.

Pharmacokinetics

After intravenous infusion of irinotecan in humans, irinotecan plasma concentrations decline in a multiexponential manner, with a mean terminal elimination half-life of about 6 to 12 hours. The mean terminal elimination half-life of the active metabolite SN-38 is about 10 to 20 hours. The half-lives of the lactone (active) forms of irinotecan and SN-38 are similar to those of total irinotecan and SN-38, as the lactone and hydroxy acid forms are in equilibrium.

Over the recommended dose range of 50 to 350 mg/m^2, the AUC of irinotecan

increases linearly with dose; the AUC of SN-38 increases less than proportionally with dose. Maximum concentrations of the active metabolite SN-38 are generally seen within 1 hour following the end of a 90-minute infusion of irinotecan. Pharmacokinetic parameters for irinotecan and SN-38 following a 90-minute infusion of irinotecan at dose levels of 125 and 340 mg/m^2 determined in two clinical studies in patients with solid tumors are summarized in Table 1:

Table 1.Summary of Mean (±Standard Deviation) Irinotecan and SN-38 Pharmacokinetic Parameters in Patients with Solid Tumors

Dose (mg/m^2)	Irinotecan					SN-38		
	C_{max} (ng/mL)	AUC_{0-24} (ng·h/mL)	$t_{1/2}$ (h)	V_z (L/m^2)	CL (L/h/m^2)	C_{max} (ng/mL)	AUC_{0-24} (ng·h/mL)	$t_{1/2}$ (h)
125 (N=64)	1,660 ±797	10,200 ±3,270	5.8[a] ±0.7	110 ±48.5	13.3 ±6.01	26.3 ±11.9	229 ±108	10.4[a] ±3.1
340 (N=6)	3,392 ±874	20,604 ±6,027	11.7[b] ±1.0	234 ±69.6	13.9 ±4.0	56.0 ±28.2	474 ±245	21.0[b] ±4.3

C_{max} - Maximum plasma concentration

AUC_{0-24} - Area under the plasma concentration-time curve from time 0 to 24 hours after the end of the 90-minute infusion

$t_{1/2}$ - Terminal elimination half-life

V_z - Volume of distribution of terminal elimination phase

CL - Total systemic clearance

[a] Plasma specimens collected for 24 hours following the end of the 90-minute infusion.

[b] Plasma specimens collected for 48 hours following the end of the 90-minute infusion. Because of the longer collection period, these values provide a more accurate reflection of the terminal elimination half-lives of irinotecan and SN-38.

Irinotecan exhibits moderate plasma protein binding (30% to 68% bound). SN-38 is highly bound to human plasma proteins (approximately 95% bound). The plasma protein to which irinotecan and SN-38 predominantly binds is albumin.

Metabolism and Excretion: The metabolic conversion of irinotecan to the active metabolite SN-38 is mediated by carboxylesterase enzymes and primarily occurs in the liver. *In vitro* studies indicate that irinotecan, SN-38 and another metabolite aminopentane carboxylic acid (APC), do not inhibit cytochrome P-450 isozymes. SN-38 is subsequently conjugated predominantly by the enzyme UDP-glucuronosyl transferase 1A1 (UGT1A1) to form a glucuronide metabolite. UGT1A1 activity is reduced in individuals with genetic polymorphisms that lead to reduced enzyme activity such as the UGT1A1*28 polymorphism. Approximately 10% of the North American population is homozygous for the UGT1A1*28 allele (also referred to as UGT1A1 7/7 genotype). In a prospective study, in which irinotecan was administered as a single-agent (350 mg/m^2) on a once-every-3-week schedule, patients with the UGT1A1 7/7 genotype had a higher exposure to SN-38 than patients with the wild-type UGT1A1 allele (UGT1A1 6/6 genotype) (See WARNINGS and DOSAGE AND ADMINISTRATION). SN-38 glucuronide had 1/50 to 1/100 the activity of SN-38 in cytotoxicity assays using two cell lines in vitro. The disposition of irinotecan has not been fully elucidated in humans. The urinary excretion of irinotecan is 11% to 20%; SN-38, <1%; and SN-38 glucuronide, 3%. The cumulative biliary and urinary excretion of irinotecan and its metabolites (SN-38 and SN-38 glucuronide) over a period of 48 hours following administration of irinotecan in

two patients ranged from approximately 25% (100 mg/m^2) to 50% (300 mg/m^2).

Pharmacokinetics in Special Populations

Geriatric: The pharmacokinetics of irinotecan administered using the weekly schedule was evaluated in a study of 183 patients that was prospectively designed to investigate the effect of age on irinotecan toxicity. Results from this trial indicate that there are no differences in the pharmacokinetics of irinotecan, SN-38, and SN-38 glucuronide in patients <65 years of age compared with patients ≥65 years of age. In a study of 162 patients that was not prospectively designed to investigate the effect of age, small (less than 18%) but statistically significant differences in dose-normalized irinotecan pharmacokinetic parameters in patients <65 years of age compared to patients ≥65 years of age were observed. Although dose-normalized AUC_{0-24} for SN-38 in patients ≥65 years of age was 11% higher than in patients <65 years of age, this difference was not statistically significant. No change in the starting dose is recommended for geriatric patients receiving the weekly dosage schedule of irinotecan. (see DOSAGE AND ADMINISTRATION).

Pediatric: See **Pediatric Use** under **PRECAUTIONS.**

Gender: The pharmacokinetics of irinotecan do not appear to be influenced by gender.

Race: The influence of race on the pharmacokinetics of irinotecan has not been evaluated.

Hepatic Insufficiency: Irinotecan clearance is diminished in patients with hepatic dysfunction while exposure to the active metabolite SN-38 is increased relative to that in patients with normal hepatic function. The magnitude of these effects is proportional to the degree of liver impairment as measured by elevations in total bilirubin and transaminase concentrations. However, the tolerability of irinotecan in patients with hepatic dysfunction (bilirubin greater than 2 mg/dl) has not been assessed sufficiently, and no recommendations for dosing can be made (see DOSAGE AND ADMINISTRATION and PRECAUTIONS: Patients at Particular Risk Sections).

Renal Insufficiency: The influence of renal insufficiency on the pharmacokinetics of irinotecan has not been evaluated. Therefore, caution should be undertaken in patients with impaired renal function. Irinotecan is not recommended for use in patients on dialysis.

Drug-Drug Interactions

5-fluorouracil (5-FU) and leucovorin (LV): In a phase 1 clinical study involving irinotecan, 5-fluorouracil (5-FU), and leucovorin (LV) in 26 patients with solid tumors, the disposition of irinotecan was not substantially altered when the drugs were co-administered. Although the C_{max} and AUC_{0-24} of SN-38, the active metabolite, were reduced (by 14% and 8%, respectively) when irinotecan was followed by 5-FU and LV administration compared with when irinotecan was given alone, this sequence of administration was used in the combination trials and is recommended (see DOSAGE AND ADMINISTRATION). Formal in vivo or in vitro drug interaction studies to evaluate the influence of irinotecan on the disposition of 5-FU and LV have not been conducted.

Anticonvulsants: Exposure to irinotecan and its active metabolite SN-38 is substantially reduced in adult and pediatric patients concomitantly receiving the CYP3A4 enzyme-inducing anticonvulsants phenytoin, phenobarbital or

carbamazepine. The appropriate starting dose for patients taking these anticonvulsants has not been formally defined. The following drugs are also CYP3A4 inducers: rifampin, rifabutin. For patients requiring anticonvulsant treatment, consideration should be given to substituting non-enzyme inducing anticonvulsants at least 2 weeks prior to initiation of irinotecan therapy. Dexamethasone does not appear to alter the pharmacokinetics of irinotecan.

St. John's Wort: St. John's Wort is an inducer of CYP3A4 enzymes. Exposure to the active metabolite SN-38 is reduced in patients receiving concomitant St. John's Wort. St. John's Wort should be discontinued at least 2 weeks prior to the first cycle of irinotecan, and St. John's Wort is contraindicated during irinotecan therapy.

Ketoconazole: Ketoconazole is a strong inhibitor of CYP3A4 enzymes. Patients receiving concomitant ketoconazole have increased exposure to irinotecan and its active metabolite SN-38. Patients should discontinue ketoconazole at least 1 week prior to starting irinotecan therapy and ketoconazole is contraindicated during irinotecan therapy.

Neuromuscular blocking agents. Interaction between irinotecan and neuromuscular blocking agents cannot be ruled out. Irinotecan has anticholinesterase activity, which may prolong the neuromuscular blocking effects of suxamethonium and the neuromuscular blockade of non-depolarizing drugs may be antagonized.

Atazanavir sulfate: Coadministration of atazanavir sulfate, a CYP3A4 and UGT1A1 inhibitor has the potential to increase systemic exposure to SN-38, the active metabolite of irinotecan. Physicians should take this into consideration when co-administering these drugs.

CLINICAL STUDIES

Irinotecan has been studied in clinical trials in combination with 5-fluorouracil (5-FU) and leucovorin (LV) and as a single agent (see DOSAGE AND ADMINISTRATION). When given as a component of combination-agent treatment, irinotecan was either given with a weekly schedule of bolus 5-FU/LV or with an every-2-week schedule of infusional 5-FU/LV. Weekly and a once-every-3-week dosage schedules were used for the single-agent irinotecan studies. Clinical studies of combination and single-agent use are described below.

First-Line Therapy in Combination with 5-FU/LV for the Treatment of Metastatic Colorectal Cancer

Two phase 3, randomized, controlled, multinational clinical trials support the use of CAMPTOSAR Injection as first-line treatment of patients with metastatic carcinoma of the colon or rectum. In each study, combinations of irinotecan with 5-FU and LV were compared with 5-FU and LV alone. Study 1 compared combination irinotecan/bolus 5-FU/LV therapy given weekly with a standard bolus regimen of 5-FU/LV alone given daily for 5 days every 4 weeks; an irinotecan-alone treatment arm given on a weekly schedule was also included. Study 2 evaluated two different methods of administering infusional 5-FU/LV, with or without irinotecan. In both studies, concomitant medications such as antiemetics, atropine, and loperamide were given to patients for prophylaxis and/or management of symptoms from treatment. In Study 2, a 7-day course of fluoroquinolone antibiotic prophylaxis was given in patients whose diarrhea persisted for

greater than 24 hours despite loperamide or if they developed a fever in addition to diarrhea. Treatment with oral fluoroquinolone was also initiated in patients who developed an absolute neutrophil count (ANC) <500/mm^3, even in the absence of fever or diarrhea. Patients in both studies also received treatment with intravenous antibiotics if they had persistent diarrhea or fever or if ileus developed.

In both studies, the combination of irinotecan/5-FU/LV therapy resulted in significant improvements in objective tumor response rates, time to tumor progression, and survival when compared with 5-FU/LV alone. These differences in survival were observed in spite of second-line therapy in a majority of patients on both arms, including crossover to irinotecan-containing regimens in the control arm. Patient characteristics and major efficacy results are shown in Table 2.

Table 2. Combination Dosage Schedule: Study Results

	Study 1			Study 2	
	Irinotecan + Bolus 5-FU/LV weekly x 4 q 6 weeks	**Bolus 5-FU/LV daily x 5 q 4 weeks**	**Irinotecan weekly x 4 q 6 weeks**	**Irinotecan + Infusional 5-FU/LV**	**Infusional 5-FU/LV**
Number of Patients	231	226	226	198	187
Demographics and Treatment Administration					
Female/Male (%)	34/65	45/54	35/64	33/67	47/53
Median Age in years (range)	62 (25-85)	61 (19-85)	61 (30-87)	62 (27-75)	59 (24-75)
Performance Status (%)					
0	39	41	46	51	51
1	46	45	46	42	41
2	15	13	8	7	8
Primary Tumor (%)					
Colon	81	85	84	55	65
Rectum	17	14	15	45	35
Median Time from Diagnosis to Randomization (months, range)	1.9 (0-161)	1.7 (0-203)	1.8 (0.1-185)	4.5 (0-88)	2.7 (0-104)
Prior Adjuvant 5-FU Therapy (%)					
No	89	92	90	74	76
Yes	11	8	10	26	24
Median Duration of Study Treatment[a] (months)	5.5	4.1	3.9	5.6	4.5
Median Relative Dose Intensity (%)[a]					
Irinotecan	72	—	75	87	—
5-FU	71	86	—	86	93
Efficacy Results					
Confirmed Objective Tumor Response Rate[b] (%)	39	21 ($p<0.0001$)[c]	18	35	22 ($p<0.005$)[c]
Median Time to Tumor Progression[d] (months)	7.0	4.3 ($p=0.004$)[d]	4.2	6.7	4.4 ($p<0.001$)[d]
Median Survival (months)	14.8	12.6 ($p<0.05$)[d]	12.0	17.4	14.1 ($p<0.05$)[d]

[a] Study 1: N=225 (irinotecan/5-FU/LV),N=219 (5-FU/LV),N=223 (irinotecan)
Study 2: N=199 (irinotecan/5-FU/LV),N=186 (5-FU/LV)

[b] Confirmed $\geq$ 4 to 6 weeks after first evidence of objective response

[c] Chi-square test

[d] Log-rank test

Improvement was noted with irinotecan-based combination therapy relative to 5-FU/LV when response rates and time to tumor progression were examined across the following demographic and disease-related subgroups (age, gender, ethnic origin, performance status, extent of organ involvement with cancer, time from diagnosis of cancer, prior adjuvant therapy, and baseline laboratory abnormalities). Figures 1 and 2 illustrate the

Kaplan-Meier survival curves for the comparison of irinotecan/5-FU/LV versus 5-FU/LV in Studies 1 and 2, respectively.

Figure 1. Survival
First-Line Irinotecan/5-FU/LV vs 5-FU/LV
Study 1

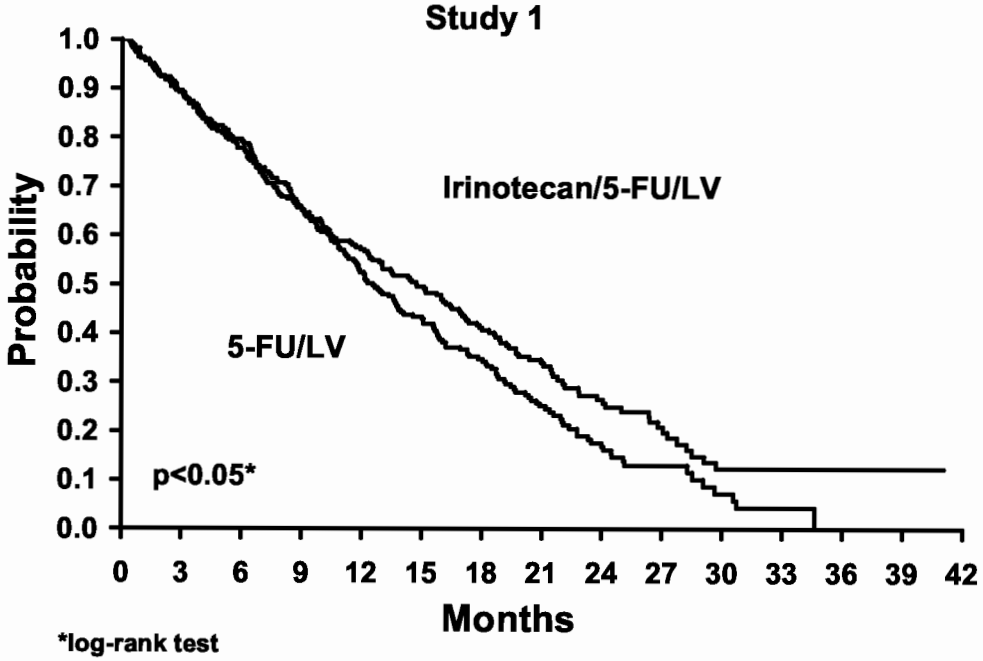
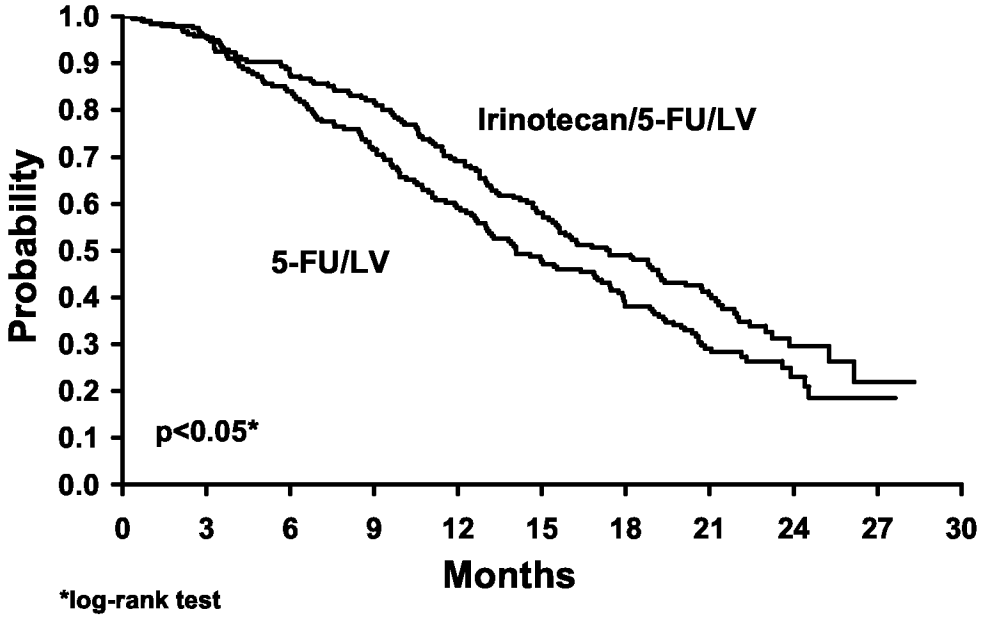


Figure 2. Survival
First-Line Irinotecan/5-FU/LV vs 5-FU/LV
Study 2



After 5-FU-Based Treatment

Weekly Dosage Schedule

Data from three open-label, single-agent, clinical studies, involving a total of 304 patients in 59 centers, support the use of CAMPTOSAR in the treatment of patients with metastatic cancer of the colon or rectum that has recurred or progressed following treatment with 5-FU-based therapy. These studies were designed to evaluate tumor response rate and do not provide information on actual clinical benefit, such as effects on survival and disease-related symptoms. In each study, CAMPTOSAR was administered in repeated 6-week cycles consisting of a 90-minute intravenous infusion once weekly for 4 weeks, followed by a 2-week rest period. Starting doses of CAMPTOSAR in these trials were 100, 125, or 150 mg/m^2, but the 150-mg/m^2 dose was poorly tolerated (due to unacceptably high rates of grade 4 late diarrhea and febrile neutropenia). Study 1 enrolled 48 patients and was conducted by a single investigator at several regional hospitals. Study 2 was a multicenter study conducted by the North Central Cancer Treatment Group. All 90 patients enrolled in Study 2 received a starting dose of 125 mg/m^2. Study 3 was a multicenter study that enrolled 166 patients from 30 institutions. The initial dose in Study 3 was 125 mg/m^2 but was reduced to 100 mg/m^2 because the toxicity seen at the 125-mg/m^2 dose was perceived to be greater than that seen in previous studies. All patients in these studies had metastatic colorectal cancer, and the majority had disease that recurred or progressed following a 5-FU-based regimen administered for metastatic disease. The results of the individual studies are shown in Table 3.

Table 3. Weekly Dosage Schedule: Study Results

	Study			
	1	2	3	
Number of Patients	48	90	64	102
Starting Dose (mg/m^2/wk x 4)	125[a]	125	125	100
Demographics and Treatment Administration				
Female/Male (%)	46/54	36/64	50/50	51/49
Median Age in years (range)	63 (29-78)	63 (32-81)	61 (42-84)	64 (25-84)
Ethnic Origin (%)				
White	79	96	81	91
African American	12	4	11	5
Hispanic	8	0	8	2
Oriental/Asian	0	0	0	2
Performance Status (%)				
0	60	38	59	44
1	38	48	33	51
2	2	14	8	5
Primary Tumor (%)				
Colon	100	71	89	87
Rectum	0	29	11	8
Unknown	0	0	0	5
Prior 5-FU Therapy (%)				
For Metastatic Disease	81	66	73	68
≤ 6 months after Adjuvant	15	7	27	28
> 6 months after Adjuvant	2	16	0	2
Classification Unknown	2	12	0	3
Prior Pelvic/Abdominal Irradiation (%)				
Yes	3	29	0	0
Other	0	9	2	4
None	97	62	98	96
Duration of Treatment with CAMPTOSAR (median, months)	5	4	4	3
Relative Dose Intensity[b] (median %)	74	67	73	81
Efficacy				
Confirmed Objective Response Rate (%)[c] (95% CI)	21 (9.3 - 32.3)	13 (6.3 - 20.4)	14 (5.5 - 22.6)	9 (3.3 - 14.3)
Time to Response (median, months)	2.6	1.5	2.8	2.8
Response Duration (median, months)	6.4	5.9	5.6	6.4
Survival (median, months)	10.4	8.1	10.7	9.3
1-Year Survival (%)	46	31	45	43

[a] Nine patients received 150 mg/m^2 as a starting dose; two (22.2%) responded to CAMPTOSAR.

[b] Relative dose intensity for CAMPTOSAR based on planned dose intensity of 100, 83.3, and 66.7 mg/m^2/wk corresponding with 150, 125, and 100 mg/m^2 starting doses, respectively.

[c] Confirmed ≥ 4 to 6 weeks after first evidence of objective response.

In the intent-to-treat analysis of the pooled data across all three studies, 193 of the 304 patients began therapy at the recommended starting dose of 125 mg/m^2. Among these 193 patients, 2 complete and 27 partial responses were observed, for an overall response rate of 15.0% (95% Confidence Interval [CI], 10.0% to 20.1%) at this starting dose. A considerably lower response rate was seen with a starting dose of 100 mg/m^2. The majority of responses were observed within the first two cycles of therapy, but responses did occur in later cycles of treatment (one response was observed after the eighth cycle). The median response duration for patients beginning therapy at 125 mg/m^2 was 5.8 months (range, 2.6 to 15.1 months). Of the 304 patients treated in the three studies, response rates to CAMPTOSAR were similar in males and females and among patients older and younger than 65 years. Rates were also similar in patients with cancer of the colon or cancer of the rectum and in patients with single and multiple metastatic sites. The response rate was 18.5% in patients with a performance status of 0 and 8.2% in patients with a performance status of 1 or 2. Patients with a performance status of 3 or 4 have not been studied. Over half of the patients responding to CAMPTOSAR had not responded to prior 5-FU. Patients who had received previous irradiation to the pelvis responded to CAMPTOSAR at approximately the same rate as those who had not previously received irradiation.

Once-Every-3-Week Dosage Schedule

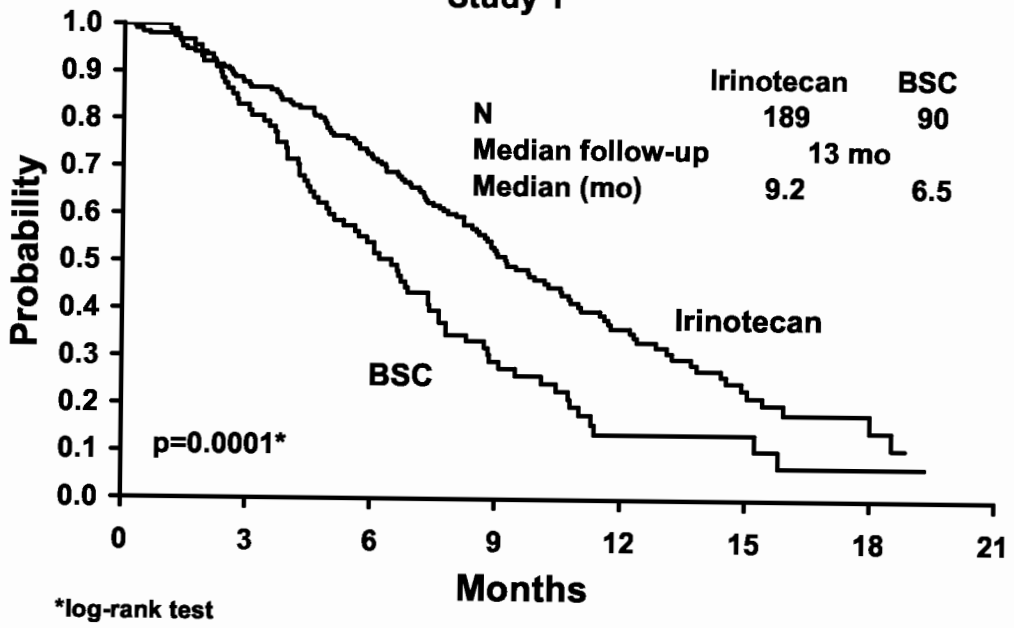
Single-Arm Studies: Data from an open-label, single-agent, single-arm, multicenter, clinical study involving a total of 132 patients support a once every-3-week dosage schedule of irinotecan in the treatment of patients with metastatic cancer of the colon or rectum that recurred or progressed following treatment with 5-FU. Patients received a starting dose of 350 mg/m^2 given by 30-minute intravenous infusion once every 3 weeks. Among the 132 previously treated patients in this trial, the intent-to-treat response rate was 12.1% (95% CI, 7.0% to 18.1%).

Randomized Trials: Two multicenter, randomized, clinical studies further support the use of irinotecan given by the once-every-3-week dosage schedule in patients with metastatic colorectal cancer whose disease has recurred or progressed following prior 5-FU therapy. In the first study, second-line irinotecan therapy plus best supportive care was compared with best supportive care alone. In the second study, second-line irinotecan therapy was compared with infusional 5-FU-based therapy. In both studies, irinotecan was administered intravenously at a starting dose of 350 mg/m^2 over 90 minutes once every 3 weeks. The starting dose was 300 mg/m^2 for patients who were 70 years and older or who had a performance status of 2. The highest total dose permitted was 700 mg. Dose reductions and/or administration delays were permitted in the event of severe hematologic and/or nonhematologic toxicities while on treatment. Best supportive care was provided to patients in both arms of Study 1 and included antibiotics, analgesics, corticosteroids, transfusions, psychotherapy, or any other symptomatic therapy as clinically indicated. In both studies, concomitant medications such as antiemetics, atropine, and loperamide were given to patients for prophylaxis and/or management of symptoms from treatment. If late diarrhea persisted for greater than 24 hours despite loperamide, a 7-day course of fluoroquinolone antibiotic prophylaxis was given. Patients in the control arm of the second study received one of the following 5-FU regimens: (1)

LV, 200 mg/m^2 IV over 2 hours; followed by 5-FU, 400 mg/m^2 IV bolus; followed by 5-FU, 600 mg/m^2 continuous IV infusion over 22 hours on days 1 and 2 every 2 weeks; (2) 5-FU, 250 to 300 mg/m^2/day protracted continuous IV infusion until toxicity; (3) 5-FU, 2.6 to 3 g/m^2 IV over 24 hours every week for 6 weeks with or without LV, 20 to 500 mg/m^2/day every week IV for 6 weeks with 2-week rest between cycles. Patients were to be followed every 3 to 6 weeks for 1 year.

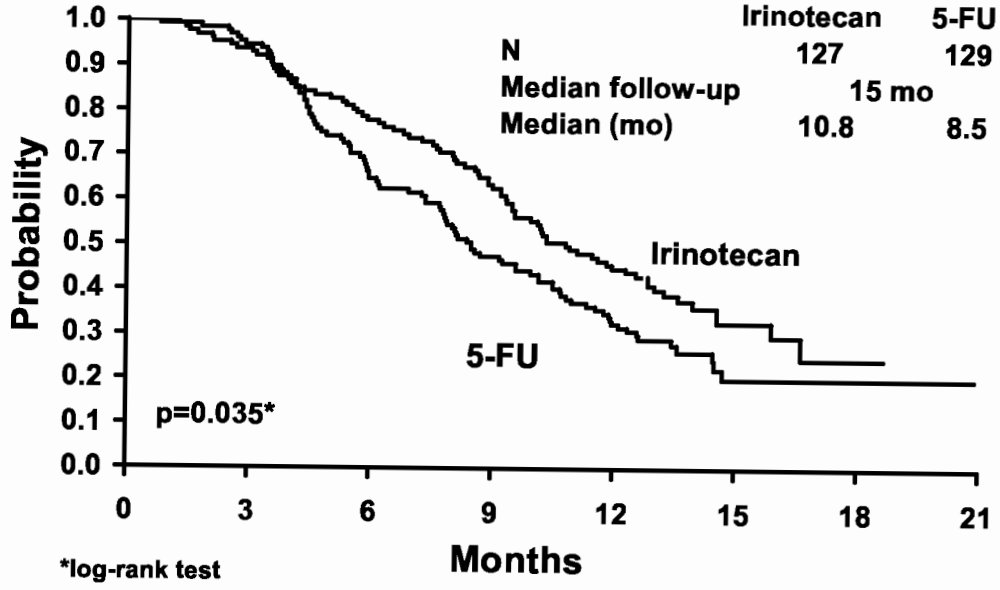
A total of 535 patients were randomized in the two studies at 94 centers. The primary endpoint in both studies was survival. The studies demonstrated a significant overall survival advantage for irinotecan compared with best supportive care (p=0.0001) and infusional 5-FU-based therapy (p=0.035) as shown in Figures 3 and 4. In Study 1, median survival for patients treated with irinotecan was 9.2 months compared with 6.5 months for patients receiving best supportive care. In Study 2, median survival for patients treated with irinotecan was 10.8 months compared with 8.5 months for patients receiving infusional 5-FU-based therapy. Multiple regression analyses determined that patients' baseline characteristics also had a significant effect on survival. When adjusted for performance status and other baseline prognostic factors, survival among patients treated with irinotecan remained significantly longer than in the control populations (p=0.001 for Study 1 and p=0.017 for Study 2). Measurements of pain, performance status, and weight loss were collected prospectively in the two studies; however, the plan for the analysis of these data was defined retrospectively. When comparing irinotecan with best supportive care in Study 1, this analysis showed a statistically significant advantage for irinotecan, with longer time to development of pain (6.9 months versus 2.0 months), time to performance status deterioration (5.7 months versus 3.3 months), and time to > 5% weight loss (6.4 months versus 4.2 months). Additionally, 33.3% (33/99) of patients with a baseline performance status of 1 or 2 showed an improvement in performance status when treated with irinotecan versus 11.3% (7/62) of patients receiving best supportive care (p=0.002). Because of the inclusion of patients with non-measurable disease, intent-to-treat response rates could not be assessed.

Figure 3. Survival
Second-Line Irinotecan vs Best Supportive Care (BSC)
Study 1



*log-rank test

Figure 4. Survival
Second-Line Irinotecan vs Infusional 5-FU
Study 2



*log-rank test

In the two randomized studies, the EORTC QLQ-C30 instrument was utilized. At the start of each cycle of therapy, patients completed a questionnaire consisting of 30 questions, such as "Did pain interfere with daily activities?" (1 = Not at All, to 4 = Very Much) and "Do you have any trouble taking a long walk?" (Yes or No). The answers from the 30 questions were converted into 15 subscales, that were scored from 0 to 100, and the global health status subscale that was derived from two questions about the patient's sense of general well being in the past week. In addition to the global health status subscale, there were five functional (i.e., cognitive, emotional, social, physical, role) and nine symptom (i.e., fatigue, appetite loss, pain assessment, insomnia, constipation, dyspnea, nausea/vomiting, financial impact, diarrhea) subscales. The results as summarized in Table 5 are based on patients' worst post-baseline scores. In Study 1, a multivariate analysis and univariate analyses of the individual subscales were performed and corrected for multivariate testing. Patients receiving irinotecan reported significantly better results for the global health status, on two of five functional subscales, and on four of nine symptom subscales. As expected, patients receiving irinotecan noted significantly more diarrhea than those receiving best supportive care. In Study 2, the multivariate analysis on all 15 subscales did not indicate a statistically significant difference between irinotecan and infusional 5-FU.

Table 4. Once-Every-3-Week Dosage Schedule: Study Results

	Study 1		Study 2	
	Irinotecan	BSC[a]	Irinotecan	5-FU
Number of Patients	189	90	127	129
Demographics and Treatment Administration				
Female/Male (%)	32/68	42/58	43/57	35/65
Median Age in years (range)	59 (22-75)	62 (34-75)	58 (30-75)	58 (25-75)
Performance Status (%)				
0	47	31	58	54
1	39	46	35	43
2	14	23	8	3
Primary Tumor (%)				
Colon	55	52	57	62
Rectum	45	48	43	38
Prior 5-FU Therapy (%)				
For Metastatic Disease	70	63	58	68
As Adjuvant Treatment	30	37	42	32
Prior Irradiation (%)	26	27	18	20
Duration of Study Treatment (median, months) (Log-rank test)	4.1	--	4.2 (p=0.02)	2.8
Relative Dose Intensity (median %)[b]	94	--	95	81-99
Survival				
Survival (median, months) (Log-rank test)	9.2 (p=0.0001)	6.5	10.8 (p=0.035)	8.5

[a] BSC = best supportive care

[b] Relative dose intensity for irinotecan based on planned dose intensity of 116.7 and 100 mg/m^2/wk corresponding with 350 and 300 mg/m^2 starting doses, respectively.

Table 5. EORTC QLQ-C30: Mean Worst Post-Baseline Score[a]

QLQ-C30 Subscale	Study 1			Study 2		
	Irinotecan	BSC	p-value	Irinotecan	5-FU	p-value
Global Health Status	47	37	0.03	53	52	0.9
Functional Scales						
Cognitive	77	68	0.07	79	83	0.9
Emotional	68	64	0.4	64	68	0.9
Social	58	47	0.06	65	67	0.9
Physical	60	40	0.0003	66	66	0.9
Role	53	35	0.02	54	57	0.9
Symptom Scales						
Fatigue	51	63	0.03	47	46	0.9
Appetite Loss	37	57	0.0007	35	38	0.9
Pain Assessment	41	56	0.009	38	34	0.9
Insomnia	39	47	0.3	39	33	0.9
Constipation	28	41	0.03	25	19	0.9
Dyspnea	31	40	0.2	25	24	0.9
Nausea/Vomiting	27	29	0.5	25	16	0.09
Financial Impact	22	26	0.5	24	15	0.3
Diarrhea	32	19	0.01	32	22	0.2

[a] For the five functional subscales and global health status subscale, higher scores imply better functioning, whereas, on the nine symptom subscales, higher scores imply more severe symptoms. The subscale scores of each patient were collected at each visit until the patient dropped out of the study.

INDICATIONS AND USAGE

CAMPTOSAR Injection is indicated as a component of first-line therapy in combination with 5-fluorouracil and leucovorin for patients with metastatic carcinoma of the colon or rectum. CAMPTOSAR is also indicated for patients with metastatic carcinoma of the colon or rectum whose disease has recurred or progressed following initial fluorouracil-based therapy.

CONTRAINDICATIONS

CAMPTOSAR Injection is contraindicated in patients with a known hypersensitivity to the drug or its excipients.

WARNINGS

General

Outside of a well-designed clinical study, CAMPTOSAR Injection should not be used in combination with the "Mayo Clinic" regimen of 5-FU/LV (administration for 4-5 consecutive days every 4 weeks) because of reports of increased toxicity, including toxic deaths. CAMPTOSAR should be used as recommended (see DOSAGE AND ADMINISTRATION, Table 11).

In patients receiving either irinotecan/5-FU/LV or 5-FU/LV in the clinical trials, higher rates of hospitalization, neutropenic fever, thromboembolism, first-cycle treatment discontinuation, and early deaths were observed in patients with a baseline performance status of 2 than in patients with a baseline performance status of 0 or 1.

Diarrhea

CAMPTOSAR can induce both early and late forms of diarrhea that appear to be mediated by different mechanisms. Early diarrhea (occurring during or shortly after infusion of CAMPTOSAR) is cholinergic in nature. It is usually transient and only infrequently is severe. It may be accompanied by symptoms of rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, and intestinal hyperperistalsis that

can cause abdominal cramping. Early diarrhea and other cholinergic symptoms may be prevented or ameliorated by administration of atropine (see PRECAUTIONS, General, for dosing recommendations for atropine).

Late diarrhea (generally occurring more than 24 hours after administration of CAMPTOSAR) can be life threatening since it may be prolonged and may lead to dehydration, electrolyte imbalance, or sepsis. Late diarrhea should be treated promptly with loperamide (see PRECAUTIONS, Information for Patients, for dosing recommendations for loperamide). Patients with diarrhea should be carefully monitored, should be given fluid and electrolyte replacement if they become dehydrated, and should be given antibiotic support if they develop ileus, fever, or severe neutropenia. After the first treatment, subsequent weekly chemotherapy treatments should be delayed in patients until return of pretreatment bowel function for at least 24 hours without need for anti-diarrhea medication. If grade 2, 3, or 4 late diarrhea occurs subsequent doses of CAMPTOSAR should be decreased within the current cycle (see DOSAGE AND ADMINISTRATION).

Neutropenia

Deaths due to sepsis following severe neutropenia have been reported in patients treated with CAMPTOSAR. Neutropenic complications should be managed promptly with antibiotic support (see PRECAUTIONS). Therapy with CAMPTOSAR should be temporarily omitted during a cycle of therapy if neutropenic fever occurs or if the absolute neutrophil count drops $<1000/mm^3$. After the patient recovers to an absolute neutrophil count $\geq 1000/mm^3$, subsequent doses of CAMPTOSAR should be reduced depending upon the level of neutropenia observed (see DOSAGE AND ADMINISTRATION).

Routine administration of a colony-stimulating factor (CSF) is not necessary, but physicians may wish to consider CSF use in individual patients experiencing significant neutropenia.

Patients with Reduced UGT1A1 Activity

Individuals who are homozygous for the UGT1A1*28 allele (UGT1A1 7/7 genotype) are at increased risk for neutropenia following initiation of CAMPTOSAR treatment.

In a study of 66 patients who received single-agent CAMPTOSAR (350 mg/m^2 once-every-3-weeks), the incidence of grade 4 neutropenia in patients homozygous for the UGT1A1*28 allele was 50%, and in patients heterozygous for this allele (UGT1A1 6/7 genotype) the incidence was 12.5%. No grade 4 neutropenia was observed in patients homozygous for the wild-type allele (UGT1A1 6/6 genotype).

In a prospective study (n=250) to investigate the role of UGT1A1*28 polymorphism in the development of toxicity in patients treated with CAMPTOSAR (180 mg/m^2) in combination with infusional 5-FU/LV, the incidence of grade 4 neutropenia in patients homozygous for the UGT1A1*28 allele was 4.5%, and in patients heterozygous for this allele the incidence was 5.3%. Grade 4 neutropenia was observed in 1.8% of patients homozygous for the wild-type allele.

In another study in which 109 patients were treated with CAMPTOSAR (100-125 mg/m^2) in combination with bolus 5-FU/LV, the incidence of grade 4 neutropenia in patients homozygous for the UGT1A1*28 allele was 18.2%, and in patients heterozygous for this allele the incidence was 11.1%. Grade 4 neutropenia was observed in 6.8% of patients homozygous for the wild-type allele.

When administered in combination with other agents, or as a single-agent, a reduction in the starting dose by at least one level of CAMPTOSAR should be considered for patients known to be homozygous for the UGT1A1*28 allele. However, the precise dose reduction in this patient population is not known and subsequent dose modifications should be considered based on individual patient tolerance to treatment (see DOSAGE AND ADMINISTRATION and PRECAUTIONS, Laboratory Tests).

Hypersensitivity

Hypersensitivity reactions including severe anaphylactic or anaphylactoid reactions have been observed.

Colitis/Ileus

Cases of colitis complicated by ulceration, bleeding, ileus, and infection have been observed. Patients experiencing ileus should receive prompt antibiotic support (see PRECAUTIONS).

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.

Thromboembolism

Thromboembolic events have been observed in patients receiving irinotecan-containing regimens; the specific cause of these events has not been determined.

Pulmonary Toxicity

Interstitial Pulmonary Disease (IPD)-like events, including fatalities, have been reported in patients receiving irinotecan (in combination and as monotherapy) for treatment of colorectal cancer and other advanced solid tumors. In the event of an acute onset of new or progressive, unexplained pulmonary symptoms such as dyspnea, cough, and fever, irinotecan and other co-prescribed chemotherapeutic agents should be interrupted pending diagnostic evaluation. If IPD is diagnosed, irinotecan and other chemotherapy should be discontinued and appropriate treatment instituted as needed (see ADVERSE REACTIONS: Overview of Adverse Events: *Respiratory*).

Pregnancy

CAMPTOSAR may cause fetal harm when administered to a pregnant woman. Radioactivity related to ^{14}C-irinotecan crosses the placenta of rats following intravenous administration of 10 mg/kg (which in separate studies produced an irinotecan C_{max} and AUC about 3 and 0.5 times, respectively, the corresponding values in patients administered 125 mg/m^2). Administration of 6 mg/kg/day intravenous irinotecan to rats (which in separate studies produced an irinotecan C_{max} and AUC about 2 and 0.2 times, respectively, the corresponding values in patients administered 125 mg/m^2) and rabbits (about one-half the recommended human weekly starting dose on a mg/m^2 basis) during the period of organogenesis, is embryotoxic as characterized by increased post-implantation loss and decreased numbers of live fetuses. Irinotecan was teratogenic in rats at doses greater than 1.2 mg/kg/day (which in separate studies produced an irinotecan C_{max} and AUC about 2/3 and 1/40th, respectively, of the corresponding values in patients administered 125 mg/m^2) and in rabbits at 6.0 mg/kg/day (about one-half the recommended human weekly starting dose on a mg/m^2 basis). Teratogenic effects included a variety of external, visceral, and skeletal abnormalities. Irinotecan

administered to rat dams for the period following organogenesis through weaning at doses of 6 mg/kg/day caused decreased learning ability and decreased female body weights in the offspring. There are no adequate and well-controlled studies of irinotecan in pregnant women. If the drug is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant while receiving treatment with CAMPTOSAR.

PRECAUTIONS

General

Care of Intravenous Site: CAMPTOSAR Injection is administered by intravenous infusion. Care should be taken to avoid extravasation, and the infusion site should be monitored for signs of inflammation. Should extravasation occur, flushing the site with sterile water and applications of ice are recommended.

Premedication with Antiemetics: Irinotecan is emetigenic. It is recommended that patients receive premedication with antiemetic agents. In clinical studies of the weekly dosage schedule, the majority of patients received 10 mg of dexamethasone given in conjunction with another type of antiemetic agent, such as a 5-HT^3 blocker (e.g., ondansetron or granisetron). Antiemetic agents should be given on the day of treatment, starting at least 30 minutes before administration of CAMPTOSAR. Physicians should also consider providing patients with an antiemetic regimen (e.g., prochlorperazine) for subsequent use as needed.

Treatment of Cholinergic Symptoms: Prophylactic or therapeutic administration of 0.25 to 1 mg of intravenous or subcutaneous atropine should be considered (unless clinically contraindicated) in patients experiencing rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, abdominal cramping, or diarrhea (occurring during or shortly after infusion of CAMPTOSAR). These symptoms are expected to occur more frequently with higher irinotecan doses.

Immunosuppressant Effects/Increased Susceptibility to Infections: Administration of live or live-attenuated vaccines in patients immunocompromised by chemotherapeutic agents including CAMPTOSAR, may result in serious or fatal infections. Avoid vaccination with a live vaccine in patients receiving irinotecan. Killed or inactivated vaccines may be administered; however, the response to such vaccines may be diminished.

Patients at Particular Risk: In patients receiving either irinotecan/5-FU/LV or 5-FU/LV in the clinical trials, higher rates of hospitalization, neutropenic fever, thromboembolism, first-cycle treatment discontinuation, and early deaths were observed in patients with a baseline performance status of 2 than in patients with a baseline performance status of 0 or 1. Patients who had previously received pelvic/abdominal radiation and elderly patients with comorbid conditions should be closely monitored.

The use of CAMPTOSAR in patients with significant hepatic dysfunction has not been established. In clinical trials of either dosing schedule, irinotecan was not administered to patients with serum bilirubin >2.0 mg/dL, or transaminase >3 times the upper limit of normal if no liver metastasis, or transaminase >5 times the upper limit of normal with liver metastasis. In clinical trials of the weekly dosage schedule, patients with modestly elevated baseline serum total bilirubin levels (1.0 to 2.0 mg/dL) had a significantly greater likelihood of experiencing first-cycle, grade 3 or 4 neutropenia than those

with bilirubin levels that were less than 1.0 mg/dL (50% [19/38] versus 18% [47/226]; p<0.001). (Also see CLINICAL PHARMACOLOGY: Pharmacokinetics in Special Populations: *Hepatic Insufficiency*). Patients with deficient glucuronidation of bilirubin, such as those with Gilbert's syndrome, may be at greater risk of myelosuppression when receiving therapy with CAMPTOSAR.

Ketoconazole, enzyme-inducing anticonvulsants and St. John's Wort are known to have drug-drug interactions with irinotecan therapy. (See Drug-Drug Interactions sub-section under CLINICAL PHARMACOLOGY)

Irinotecan commonly causes neutropenia, leucopenia, and anemia, any of which may be severe and therefore should not be used in patients with severe bone marrow failure.

Patients must not be treated with irinotecan until resolution of the bowel obstruction.

Patients with hereditary fructose intolerance should not be given CAMPTOSAR, as this product contains sorbitol.

Information for Patients

Patients and patients' caregivers should be informed of the expected toxic effects of CAMPTOSAR, particularly of its gastrointestinal complications, such as nausea, vomiting, abdominal cramping, diarrhea, and infection. Each patient should be instructed to have loperamide readily available and to begin treatment for late diarrhea (generally occurring more than 24 hours after administration of CAMPTOSAR) at the first episode of poorly formed or loose stools or the earliest onset of bowel movements more frequent than normally expected for the patient. One dosage regimen for loperamide used in clinical trials consisted of the following (Note: This dosage regimen exceeds the usual dosage recommendations for loperamide.): 4 mg at the first onset of late diarrhea and then 2 mg every 2 hours until the patient is diarrhea-free for at least 12 hours. Loperamide is not recommended to be used for more than 48 consecutive hours at these doses, because of the risk of paralytic ileus. During the night, the patient may take 4 mg of loperamide every 4 hours. Premedication with loperamide is not recommended. The use of drugs with laxative properties should be avoided because of the potential for exacerbation of diarrhea. Patients should be advised to contact their physician to discuss any laxative use.

Patients should be instructed to contact their physician or nurse if any of the following occur: diarrhea for the first time during treatment; black or bloody stools; symptoms of dehydration such as lightheadedness, dizziness, or faintness; inability to take fluids by mouth due to nausea or vomiting; inability to get diarrhea under control within 24 hours; or fever or evidence of infection.

Patients should be warned about the potential for dizziness or visual disturbances which may occur within 24 hours following the administration of CAMPTOSAR, and advised not to drive or operate machinery if these symptoms occur.

Patients should be alerted to the possibility of alopecia.

Laboratory Tests

Careful monitoring of the white blood cell count with differential, hemoglobin, and platelet count is recommended before each dose of CAMPTOSAR.

UGT1A1 Testing

A laboratory test is available to determine the UGT1A1 status of patients._Testing can detect the UGT1A1 6/6, 6/7 and 7/7 genotypes (See WARNINGS).

Drug Interactions

The adverse effects of CAMPTOSAR, such as myelosuppression and diarrhea, would be expected to be exacerbated by other antineoplastic agents having similar adverse effects.

Patients who have previously received pelvic/ abdominal irradiation are at increased risk of severe myelosuppression following the administration of CAMPTOSAR. The concurrent administration of CAMPTOSAR with irradiation has not been adequately studied and is not recommended.

Lymphocytopenia has been reported in patients receiving CAMPTOSAR, and it is possible that the administration of dexamethasone as antiemetic prophylaxis may have enhanced the likelihood of this effect. However, serious opportunistic infections have not been observed, and no complications have specifically been attributed to lymphocytopenia.

Hyperglycemia has also been reported in patients receiving CAMPTOSAR. Usually, this has been observed in patients with a history of diabetes mellitus or evidence of glucose intolerance prior to administration of CAMPTOSAR. It is probable that dexamethasone, given as antiemetic prophylaxis, contributed to hyperglycemia in some patients.

The incidence of akathisia in clinical trials of the weekly dosage schedule was greater (8.5%, 4/47 patients) when prochlorperazine was administered on the same day as CAMPTOSAR than when these drugs were given on separate days (1.3%, 1/80 patients). The 8.5% incidence of akathisia, however, is within the range reported for use of prochlorperazine when given as a premedication for other chemotherapies.

It would be expected that laxative use during therapy with CAMPTOSAR would worsen the incidence or severity of diarrhea, but this has not been studied.

In view of the potential risk of dehydration secondary to vomiting and/or diarrhea induced by CAMPTOSAR, the physician may wish to withhold diuretics during dosing with CAMPTOSAR and, certainly, during periods of active vomiting or diarrhea.

Drug-Laboratory Test Interactions

There are no known interactions between CAMPTOSAR and laboratory tests.

Carcinogenesis, Mutagenesis & Impairment of Fertility

Long-term carcinogenicity studies with irinotecan were not conducted. Rats were, however, administered intravenous doses of 2 mg/kg or 25 mg/kg irinotecan once per week for 13 weeks (in separate studies, the 25 mg/kg dose produced an irinotecan C_{max} and AUC that were about 7.0 times and 1.3 times the respective values in patients administered 125 mg/m^2 weekly) and were then allowed to recover for 91 weeks. Under these conditions, there was a significant linear trend with dose for the incidence of combined uterine horn endometrial stromal polyps and endometrial stromal sarcomas. Neither irinotecan nor SN-38 was mutagenic in the in vitro Ames assay. Irinotecan was clastogenic both in vitro (chromosome aberrations in Chinese hamster ovary cells) and in vivo (micronucleus test in mice). No significant adverse effects on fertility and general reproductive performance were observed after intravenous administration of irinotecan in doses of up to 6 mg/kg/day to rats and rabbits. However, atrophy of male reproductive organs was observed after multiple daily irinotecan doses both in rodents at 20 mg/kg (which in separate studies produced an irinotecan C_{max} and AUC about 5 and 1 times, respectively, the corresponding values in patients administered 125 mg/m^2 weekly) and

dogs at 0.4 mg/kg (which in separate studies produced an irinotecan C_{max} and AUC about one-half and 1/15th, respectively, the corresponding values in patients administered 125 mg/m^2 weekly).

Pregnancy

Pregnancy Category D—see WARNINGS.

Nursing Mothers

Radioactivity appeared in rat milk within 5 minutes of intravenous administration of radiolabeled irinotecan and was concentrated up to 65-fold at 4 hours after administration relative to plasma concentrations. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants, it is recommended that nursing be discontinued when receiving therapy with CAMPTOSAR.

Pediatric Use

The effectiveness of irinotecan in pediatric patients has not been established. Results from two open-label, single arm studies were evaluated. One hundred and seventy children with refractory solid tumors were enrolled in one phase 2 trial in which 50 mg/m^2 of irinotecan was infused for 5 consecutive days every 3 weeks. Grade 3-4 neutropenia was experienced by 54 (31.8%) patients. Neutropenia was complicated by fever in 15 (8.8%) patients. Grade 3-4 diarrhea was observed in 35 (20.6%) patients. This adverse event profile was comparable to that observed in adults. In the second phase 2 trial of 21 children with previously untreated rhabdomyosarcoma, 20 mg/m^2 of irinotecan was infused for 5 consecutive days on weeks 0, 1, 3 and 4. This single agent therapy was followed by multimodal therapy. Accrual to the single agent irinotecan phase was halted due to the high rate (28.6%) of progressive disease and the early deaths (14%). The adverse event profile was different in this study from that observed in adults; the most significant grade 3 or 4 adverse events were dehydration experienced by 6 patients (28.6%) associated with severe hypokalemia in 5 patients (23.8%) and hyponatremia in 3 patients (14.3%); in addition Grade 3-4 infection was reported in 5 patients (23.8%) (across all courses of therapy and irrespective of causal relationship).

Pharmacokinetic parameters for irinotecan and SN-38 were determined in 2 pediatric solid-tumor trials at dose levels of 50 mg/m^2 (60-min infusion, n=48) and 125 mg/m^2 (90-min infusion, n=6). Irinotecan clearance (mean ± S.D.) was 17.3 ± 6.7 L/h/m^2 for the 50mg/m^2 dose and 16.2 ± 4.6 L/h/m^2 for the 125 mg/m^2 dose, which is comparable to that in adults. Dose-normalized SN-38 AUC values were comparable between adults and children. Minimal accumulation of irinotecan and SN-38 was observed in children on daily dosing regimens [daily x 5 every 3 weeks or (daily x 5) x 2 weeks every 3 weeks].

Geriatric Use

Patients greater than 65 years of age should be closely monitored because of a greater risk of early and late diarrhea in this population (see CLINICAL PHARMACOLOGY, Pharmacokinetics in Special Populations and ADVERSE REACTIONS, Overview of Adverse Events). The starting dose of CAMPTOSAR in patients 70 years and older for the once-every-3-week-dosage schedule should be 300 mg/m^2 (see DOSAGE AND ADMINISTRATION).

ADVERSE REACTIONS

First-Line Combination Therapy

A total of 955 patients with metastatic colorectal cancer received the recommended regimens of irinotecan in combination with 5-FU/LV, 5-FU/LV alone, or irinotecan alone. In the two phase 3 studies, 370 patients received irinotecan in combination with 5-FU/LV, 362 patients received 5-FU/LV alone, and 223 patients received irinotecan alone. (See Table 11 in DOSAGE AND ADMINISTRATION for recommended combination-agent regimens.)

In Study 1, 49 (7.3%) patients died within 30 days of last study treatment: 21 (9.3%) received irinotecan in combination with 5-FU/LV, 15 (6.8%) received 5-FU/LV alone, and 13 (5.8%) received irinotecan alone. Deaths potentially related to treatment occurred in 2 (0.9%) patients who received irinotecan in combination with 5-FU/LV (2 neutropenic fever/sepsis), 3 (1.4%) patients who received 5-FU/LV alone (1 neutropenic fever/sepsis, 1 CNS bleeding during thrombocytopenia, 1 unknown) and 2 (0.9%) patients who received irinotecan alone (2 neutropenic fever). Deaths from any cause within 60 days of first study treatment were reported for 15 (6.7%) patients who received irinotecan in combination with 5-FU/LV, 16 (7.3%) patients who received 5-FU/LV alone, and 15 (6.7%) patients who received irinotecan alone. Discontinuations due to adverse events were reported for 17 (7.6%) patients who received irinotecan in combination with 5FU/LV, 14 (6.4%) patients who received 5-FU/LV alone, and 26 (11.7%) patients who received irinotecan alone.

In Study 2, 10 (3.5%) patients died within 30 days of last study treatment: 6 (4.1%) received irinotecan in combination with 5-FU/LV and 4 (2.8%) received 5-FU/LV alone. There was one potentially treatment-related death, which occurred in a patient who received irinotecan in combination with 5-FU/LV (0.7%, neutropenic sepsis). Deaths from any cause within 60 days of first study treatment were reported for 3 (2.1%) patients who received irinotecan in combination with 5-FU/LV and 2 (1.4%) patients who received 5-FU/LV alone. Discontinuations due to adverse events were reported for 9 (6.2%) patients who received irinotecan in combination with 5FU/LV and 1 (0.7%) patient who received 5-FU/LV alone.

The most clinically significant adverse events for patients receiving irinotecan-based therapy were diarrhea, nausea, vomiting, neutropenia, and alopecia. The most clinically significant adverse events for patients receiving 5-FU/LV therapy were diarrhea, neutropenia, neutropenic fever, and mucositis. In Study 1, grade 4 neutropenia, neutropenic fever (defined as grade 2 fever and grade 4 neutropenia), and mucositis were observed less often with weekly irinotecan/5-FU/LV than with monthly administration of 5-FU/LV.

Tables 6 and 7 list the clinically relevant adverse events reported in Studies 1 and 2, respectively.

Table 6. Study 1: Percent (%) of Patients Experiencing Clinically Relevant Adverse Events in Combination Therapies[a]

Adverse Event	Study 1					
	Irinotecan + Bolus 5-FU/LV weekly x 4 q 6 weeks N=225		Bolus 5-FU/LV daily x 5 q 4 weeks N=219		Irinotecan weekly x 4 q 6 weeks N=223	
	Grade 1-4	Grade 3&4	Grade 1-4	Grade 3&4	Grade 1-4	Grade 3&4
TOTAL Adverse Events	100	53.3	100	45.7	99.6	45.7
GASTROINTESTINAL						
Diarrhea						
late	84.9	22.7	69.4	13.2	83.0	31.0
grade 3	--	15.1	--	5.9	--	18.4
grade 4	--	7.6	--	7.3	--	12.6
early	45.8	4.9	31.5	1.4	43.0	6.7
Nausea	79.1	15.6	67.6	8.2	81.6	16.1
Abdominal pain	63.1	14.6	50.2	11.5	67.7	13.0
Vomiting	60.4	9.7	46.1	4.1	62.8	12.1
Anorexia	34.2	5.8	42.0	3.7	43.9	7.2
Constipation	41.3	3.1	31.5	1.8	32.3	0.4
Mucositis	32.4	2.2	76.3	16.9	29.6	2.2
HEMATOLOGIC						
Neutropenia	96.9	53.8	98.6	66.7	96.4	31.4
grade 3	--	29.8	--	23.7	--	19.3
grade 4	--	24.0	--	42.5	--	12.1
Leukopenia	96.9	37.8	98.6	23.3	96.4	21.5
Anemia	96.9	8.4	98.6	5.5	96.9	4.5
Neutropenic fever	--	7.1	--	14.6	--	5.8
Thrombocytopenia	96.0	2.6	98.6	2.7	96.0	1.7
Neutropenic infection	--	1.8	--	0	--	2.2
BODY AS A WHOLE						
Asthenia	70.2	19.5	64.4	11.9	69.1	13.9
Pain	30.7	3.1	26.9	3.6	22.9	2.2
Fever	42.2	1.7	32.4	3.6	43.5	0.4
Infection	22.2	0	16.0	1.4	13.9	0.4
METABOLIC & NUTRITIONAL						
↑ Bilirubin	87.6	7.1	92.2	8.2	83.9	7.2
DERMATOLOGIC						
Exfoliative dermatitis	0.9	0	3.2	0.5	0	0
Rash	19.1	0	26.5	0.9	14.3	0.4
Alopecia[b]	43.1	--	26.5	--	46.1	--
RESPIRATORY						
Dyspnea	27.6	6.3	16.0	0.5	22.0	2.2
Cough	26.7	1.3	18.3	0	20.2	0.4
Pneumonia	6.2	2.7	1.4	1.0	3.6	1.3
NEUROLOGIC						
Dizziness	23.1	1.3	16.4	0	21.1	1.8
Somnolence	12.4	1.8	4.6	1.8	9.4	1.3
Confusion	7.1	1.8	4.1	0	2.7	0
CARDIOVASCULAR						
Vasodilatation	9.3	0.9	5.0	0	9.0	0
Hypotension	5.8	1.3	2.3	0.5	5.8	1.7
Thromboembolic events[c]	9.3	--	11.4	--	5.4	--

[a]Severity of adverse events based on NCI CTC (version 1.0)

[b]Complete hair loss = Grade 2

[c]Includes angina pectoris, arterial thrombosis, cerebral infarct, cerebrovascular accident, deep thrombophlebitis, embolus lower extremity, heart arrest, myocardial infarct, myocardial ischemia, peripheral vascular disorder, pulmonary embolus, sudden death, thrombophlebitis, thrombosis, vascular disorder.

Table 7. Study 2: Percent (%) of Patients Experiencing Clinically Relevant Adverse Events in Combination Therapies[a]

Adverse Event	Study 2			
	Irinotecan + 5-FU/LV infusional d 1&2 q 2 weeks N= 145		5-FU/LV infusional d 1&2 q 2 weeks N=143	
	Grade 1-4	Grade 3&4	Grade 1-4	Grade 3&4
TOTAL Adverse Events	100	72.4	100	39.2
GASTROINTESTINAL				
Diarrhea				
late	72.4	14.4	44.8	6.3
grade 3	--	10.3	--	4.2
grade 4	--	4.1	--	2.1
Cholinergic syndrome[b]	28.3	1.4	0.7	0
Nausea	66.9	2.1	55.2	3.5
Abdominal pain	17.2	2.1	16.8	0.7
Vomiting	44.8	3.5	32.2	2.8
Anorexia	35.2	2.1	18.9	0.7
Constipation	30.3	0.7	25.2	1.4
Mucositis	40.0	4.1	28.7	2.8
HEMATOLOGIC				
Neutropenia	82.5	46.2	47.9	13.4
grade 3	--	36.4	--	12.7
grade 4	--	9.8	--	0.7
Leukopenia	81.3	17.4	42.0	3.5
Anemia	97.2	2.1	90.9	2.1
Neutropenic fever	--	3.4	--	0.7
Thrombocytopenia	32.6	0	32.2	0
Neutropenic infection	--	2.1	--	0
BODY AS A WHOLE				
Asthenia	57.9	9.0	48.3	4.2
Pain	64.1	9.7	61.5	8.4
Fever	22.1	0.7	25.9	0.7
Infection	35.9	7.6	33.6	3.5
METABOLIC & NUTRITIONAL				
↑ Bilirubin	19.1	3.5	35.9	10.6
DERMATOLOGIC				
Hand & foot syndrome	10.3	0.7	12.6	0.7
Cutaneous signs	17.2	0.7	20.3	0
Alopecia[c]	56.6	--	16.8	--
RESPIRATORY				
Dyspnea	9.7	1.4	4.9	0
CARDIOVASCULAR				
Hypotension	3.4	1.4	0.7	0
Thromboembolic events[d]	11.7	--	5.6	--

[a] Severity of adverse events based on NCI CTC (version 1.0)

[b] Includes rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, abdominal cramping or diarrhea (occurring during or shortly after infusion of irinotecan)

[c] Complete hair loss = Grade 2

[d] Includes angina pectoris, arterial thrombosis, cerebral infarct, cerebrovascular accident, deep thrombophlebitis, embolus lower extremity, heart arrest, myocardial infarct, myocardial ischemia, peripheral vascular disorder, pulmonary embolus, sudden death, thrombophlebitis, thrombosis, vascular disorder.

Second-Line Single-Agent Therapy

Weekly Dosage Schedule

In three clinical studies evaluating the weekly dosage schedule, 304 patients with metastatic carcinoma of the colon or rectum that had recurred or progressed following 5-FU-based therapy were treated with CAMPTOSAR. Seventeen of the patients died within 30 days of the administration of CAMPTOSAR; in five cases (1.6%, 5/304), the deaths were potentially drug-related. These five patients experienced a constellation of medical events that included known effects of CAMPTOSAR. One of these patients died of neutropenic sepsis without fever. Neutropenic fever occurred in nine (3.0%) other patients; these patients recovered with supportive care.

One hundred nineteen (39.1%) of the 304 patients were hospitalized a total of 156 times because of adverse events; 81 (26.6%) patients were hospitalized for events judged to be related to administration of CAMPTOSAR. The primary reasons for drug-related hospitalization were diarrhea, with or without nausea and/or vomiting (18.4%); neutropenia/leukopenia, with or without diarrhea and/or fever (8.2%); and nausea and/or vomiting (4.9%).

Adjustments in the dose of CAMPTOSAR were made during the cycle of treatment and for subsequent cycles based on individual patient tolerance. The first dose of at least one cycle of CAMPTOSAR was reduced for 67% of patients who began the studies at the 125-mg/m^2 starting dose. Within-cycle dose reductions were required for 32% of the cycles initiated at the 125-mg/m^2 dose level. The most common reasons for dose reduction were late diarrhea, neutropenia, and leukopenia. Thirteen (4.3%) patients discontinued treatment with CAMPTOSAR because of adverse events. The adverse events in Table 8 are based on the experience of the 304 patients enrolled in the three studies described in the CLINICAL STUDIES, Studies Evaluating the Weekly Dosage Schedule, section.

Table 8. Adverse Events Occurring in >10% of 304 Previously Treated Patients with Metastatic Carcinoma of the Colon or Rectum[a]

Body System & Event	% of Patients Reporting	
	NCI Grades 1-4	NCI Grades 3 & 4
GASTROINTESTINAL		
Diarrhea (late)[b]	88	31
7-9 stools/day (grade 3)	—	(16)
≥10 stools/day (grade 4)	—	(14)
Nausea	86	17
Vomiting	67	12
Anorexia	55	6
Diarrhea (early)[c]	51	8
Constipation	30	2
Flatulence	12	0
Stomatitis	12	1
Dyspepsia	10	0
HEMATOLOGIC		
Leukopenia	63	28
Anemia	60	7
Neutropenia	54	26
500 to <1000/mm^3 (grade 3)	—	(15)
<500/mm^3 (grade 4)	—	(12)
BODY AS A WHOLE		
Asthenia	76	12
Abdominal cramping/pain	57	16
Fever	45	1
Pain	24	2
Headache	17	1
Back pain	14	2
Chills	14	0
Minor infection[d]	14	0
Edema	10	1
Abdominal enlargement	10	0
METABOLIC & NUTRITIONAL		
↓ Body weight	30	1
Dehydration	15	4
↑ Alkaline phosphatase	13	4
↑ SGOT	10	1
DERMATOLOGIC		
Alopecia	60	NA[e]
Sweating	16	0
Rash	13	1
RESPIRATORY		
Dyspnea	22	4
↑ Coughing	17	0
Rhinitis	16	0
NEUROLOGIC		
Insomnia	19	0
Dizziness	15	0
CARDIOVASCULAR		
Vasodilation (flushing)	11	0

[a] Severity of adverse events based on NCI CTC (version 1.0)
[b] Occurring > 24 hours after administration of CAMPTOSAR
[c] Occurring ≤24 hours after administration of CAMPTOSAR
[d] Primarily upper respiratory infections
[e] Not applicable; complete hair loss = NCI grade 2

Once-Every-3-Week Dosage Schedule

A total of 535 patients with metastatic colorectal cancer whose disease had recurred or progressed following prior 5-FU therapy participated in the two phase 3 studies: 316 received irinotecan, 129 received 5-FU, and 90 received best supportive care. Eleven (3.5%) patients treated with irinotecan died within 30 days of treatment. In three cases (1%, 3/316), the deaths were potentially related to irinotecan treatment and were attributed to neutropenic infection, grade 4 diarrhea, and asthenia, respectively. One (0.8%, 1/129) patient treated with 5-FU died within 30 days of treatment; this death was attributed to grade 4 diarrhea.

Hospitalizations due to serious adverse events (whether or not related to study treatment) occurred at least once in 60% (188/316) of patients who received irinotecan, 63% (57/90) who received best supportive care, and 39% (50/129) who received 5-FU-based therapy. Eight percent of patients treated with irinotecan and 7% treated with 5-FU-based therapy discontinued treatment due to adverse events.

Of the 316 patients treated with irinotecan, the most clinically significant adverse events (all grades, 1-4) were diarrhea (84%), alopecia (72%), nausea (70%), vomiting (62%), cholinergic symptoms (47%), and neutropenia (30%). Table 9 lists the grade 3 and 4 adverse events reported in the patients enrolled to all treatment arms of the two studies described in the CLINICAL STUDIES, Studies Evaluating the Once-Every-3-Week Dosage Schedule, section.

Table 9. Percent Of Patients Experiencing Grade 3 & 4 Adverse Events In Comparative Studies Of Once-Every-3-Week Irinotecan Therapy[a]

	Study 1		Study 2	
Adverse Event	**Irinotecan N=189**	**BSC [b] N=90**	**Irinotecan N=127**	**5-FU N=129**
TOTAL Grade 3/4 Adverse Events	79	67	69	54
GASTROINTESTINAL				
Diarrhea	22	6	22	11
Vomiting	14	8	14	5
Nausea	14	3	11	4
Abdominal pain	14	16	9	8
Constipation	10	8	8	6
Anorexia	5	7	6	4
Mucositis	2	1	2	5
HEMATOLOGIC				
Leukopenia/Neutropenia	22	0	14	2
Anemia	7	6	6	3
Hemorrhage	5	3	1	3
Thrombocytopenia	1	0	4	2
Infection				
without grade 3/4 neutropenia	8	3	1	4
with grade 3/4 neutropenia	1	0	2	0
Fever				
without grade 3/4 neutropenia	2	1	2	0
with grade 3/4 neutropenia	2	0	4	2
BODY AS A WHOLE				
Pain	19	22	17	13
Asthenia	15	19	13	12
METABOLIC & NUTRITIONAL				
Hepatic [c]	9	7	9	6
DERMATOLOGIC				
Hand & foot syndrome	0	0	0	5
Cutaneous signs [d]	2	0	1	3
RESPIRATORY [e]	10	8	5	7
NEUROLOGIC [f]	12	13	9	4
CARDIOVASCULAR [g]	9	3	4	2
OTHER [h]	32	28	12	14

[a] Severity of adverse events based on NCI CTC (version 1.0)
[b] BSC = best supportive care
[c] Hepatic includes events such as ascites and jaundice
[d] Cutaneous signs include events such as rash
[e] Respiratory includes events such as dyspnea and cough
[f] Neurologic includes events such as somnolence
[g] Cardiovascular includes events such as dysrhythmias, ischemia, and mechanical cardiac dysfunction
[h] Other includes events such as accidental injury, hepatomegaly, syncope, vertigo, and weight loss

Overview of Adverse Events

Gastrointestinal: Nausea, vomiting, and diarrhea are common adverse events following treatment with CAMPTOSAR and can be severe. When observed, nausea and vomiting usually occur during or shortly after infusion of CAMPTOSAR. An increased incidence of late diarrhea was observed in two studies, one using a 3-week schedule and the other using a weekly schedule. In the clinical studies testing the every 3-week-dosage schedule, the median time to the onset of late diarrhea was 5 days after irinotecan infusion. In the clinical studies evaluating the weekly dosage schedule, the median time to onset of late diarrhea was 11 days following administration of CAMPTOSAR. For patients

starting treatment at the 125-mg/m^2 weekly dose, the median duration of any grade of late diarrhea was 3 days. Among those patients treated at the 125-mg/m^2 weekly dose who experienced grade 3 or 4 late diarrhea, the median duration of the entire episode of diarrhea was 7 days. The frequency of grade 3 or 4 late diarrhea was somewhat greater in patients starting treatment at 125 mg/m^2 than in patients given a 100-mg/m^2 weekly starting dose (34% [65/193] versus 23% [24/102]; p=0.08). The frequency of grade 3 and 4 late diarrhea by age was significantly greater in patients ≥65 years than in patients <65 years (40% [53/133] versus 23% [40/171]; p=0.002). In another study of 183 patients treated on the weekly schedule, the frequency of grade 3 or 4 late diarrhea in patients ≥65 years of age was 28.6% [26/91] and in patients <65 years of age was 23.9% [22/92].
In one study of the weekly dosage treatment, the frequency of grade 3 and 4 late diarrhea was significantly greater in male than in female patients (43% [25/58] versus 16% [5/32]; p=0.01), but there were no gender differences in the frequency of grade 3 and 4 late diarrhea in the other two studies of the weekly dosage treatment schedule. Colonic ulceration, sometimes with gastrointestinal bleeding, has been observed in association with administration of CAMPTOSAR.
Hematology: CAMPTOSAR commonly causes neutropenia, leukopenia (including lymphocytopenia), and anemia. Serious thrombocytopenia is uncommon. When evaluated in the trials of weekly administration, the frequency of grade 3 and 4 neutropenia was significantly higher in patients who received previous pelvic/abdominal irradiation than in those who had not received such irradiation (48% [13/27] versus 24% [67/277]; p=0.04). In these same studies, patients with baseline serum total bilirubin levels of 1.0 mg/dL or more also had a significantly greater likelihood of experiencing first-cycle grade 3 or 4 neutropenia than those with bilirubin levels that were less than 1.0 mg/dL (50% [19/38] versus 18% [47/266]; p<0.001). There were no significant differences in the frequency of grade 3 and 4 neutropenia by age or gender. In the clinical studies evaluating the weekly dosage schedule, neutropenic fever (concurrent NCI grade 4 neutropenia and fever of grade 2 or greater) occurred in 3% of the patients; 6% of patients received G-CSF for the treatment of neutropenia. NCI grade 3 or 4 anemia was noted in 7% of the patients receiving weekly treatment; blood transfusions were given to 10% of the patients in these trials.
Body as a Whole: Asthenia, fever, and abdominal pain are generally the most common events of this type.
Cholinergic Symptoms: Patients may have cholinergic symptoms of rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, and intestinal hyperperistalsis that can cause abdominal cramping and early diarrhea. If these symptoms occur, they manifest during or shortly after drug infusion. They are thought to be related to the anticholinesterase activity of the irinotecan parent compound and are expected to occur more frequently with higher irinotecan doses.
Hepatic: In the clinical studies evaluating the weekly dosage schedule, NCI grade 3 or 4 liver enzyme abnormalities were observed in fewer than 10% of patients. These events typically occur in patients with known hepatic metastases.
Dermatologic: Alopecia has been reported during treatment with CAMPTOSAR.

Rashes have also been reported but did not result in discontinuation of treatment.
Respiratory: Severe pulmonary events are infrequent. In the clinical studies evaluating the weekly dosage schedule, NCI grade 3 or 4 dyspnea was reported in 4% of patients. Over half the patients with dyspnea had lung metastases; the extent to which malignant pulmonary involvement or other preexisting lung disease may have contributed to dyspnea in these patients is unknown.

Interstitial pulmonary disease presenting as pulmonary infiltrates is uncommon during irinotecan therapy. Interstitial pulmonary disease can be fatal. Risk factors possibly associated with the development of interstitial pulmonary disease include pre-existing lung disease, use of pneumotoxic drugs, radiation therapy, and colony stimulating factors. Patients with risk factors should be closely monitored for respiratory symptoms before and during irinotecan therapy (see WARNINGS).

Neurologic: Insomnia and dizziness can occur, but are not usually considered to be directly related to the administration of CAMPTOSAR. Dizziness may sometimes represent symptomatic evidence of orthostatic hypotension in patients with dehydration.

Cardiovascular: Vasodilation (flushing) may occur during administration of CAMPTOSAR. Bradycardia may also occur, but has not required intervention. These effects have been attributed to the cholinergic syndrome sometimes observed during or shortly after infusion of CAMPTOSAR. Thromboembolic events have been observed in patients receiving CAMPTOSAR; the specific cause of these events has not been determined.

Other Non-U.S. Clinical Trials

Irinotecan has been studied in over 1100 patients in Japan. Patients in these studies had a variety of tumor types, including cancer of the colon or rectum, and were treated with several different doses and schedules. In general, the types of toxicities observed were similar to those seen in U.S. trials with CAMPTOSAR. There is some information from Japanese trials that patients with considerable ascites or pleural effusions were at increased risk for neutropenia or diarrhea. A potentially life-threatening pulmonary syndrome, consisting of dyspnea, fever, and a reticulonodular pattern on chest x-ray, was observed in a small percentage of patients in early Japanese studies. The contribution of irinotecan to these preliminary events was difficult to assess because these patients also had lung tumors and some had preexisting nonmalignant pulmonary disease. As a result of these observations, however, clinical studies in the United States have enrolled few patients with compromised pulmonary function, significant ascites, or pleural effusions.

Post-Marketing Experience

The following events have been identified during postmarketing use of CAMPTOSAR in clinical practice. Myocardial ischemic events have been observed following irinotecan therapy (See also Table 7, thromboembolic events) Infrequent cases of ulcerative and ischemic colitis have been observed. This can be complicated by ulceration, bleeding, ileus, obstruction, and infection, including typhlitis. Patients experiencing ileus should receive prompt antibiotic support (see PRECAUTIONS). Cases of megacolon, intestinal perforation, symptomatic pancreatitis, and asymptomatic pancreatic enzyme elevation have been reported.

Hypersensitivity reactions including severe anaphylactic or anaphylactoid reactions have also been observed (see WARNINGS).

Cases of hyponatremia mostly related with diarrhea and vomiting have been reported. Increases in serum levels of transaminases (i.e., AST and ALT) in the absence of progressive liver metastasis; transient increase of amylase and occasionally transient increase of lipase have been reported.

Infrequent cases of renal insufficiency including acute renal failure, hypotension or circulatory failure have been observed in patients who experienced episodes of dehydration associated with diarrhea and/or vomiting, or sepsis (see WARNINGS).

Early effects such as muscular contraction or cramps and paresthesia have been reported.

Hiccups have been reported.

Transient dysarthria has been reported in patients treated with CAMPTOSAR; in some cases, the event was attributed to the cholinergic syndrome observed during or shortly after infusion of irinotecan.

OVERDOSAGE

In U.S. phase 1 trials, single doses of up to 345 mg/m^2 of irinotecan were administered to patients with various cancers. Single doses of up to 750 mg/m^2 of irinotecan have been given in non-U.S. trials. The adverse events in these patients were similar to those reported with the recommended dosage and regimen. There have been reports of overdosage at doses up to approximately twice the recommended therapeutic dose, which may be fatal. The most significant adverse reactions reported were severe neutropenia and severe diarrhea. There is no known antidote for overdosage of CAMPTOSAR. Maximum supportive care should be instituted to prevent dehydration due to diarrhea and to treat any infectious complications.

DOSAGE AND ADMINISTRATION

Combination-Agent Dosage

Dosage Regimens

CAMPTOSAR Injection in Combination with 5-Fluorouracil (5-FU) and Leucovorin (LV)

CAMPTOSAR should be administered as an intravenous infusion over 90 minutes (see Preparation of Infusion Solution). For all regimens, the dose of LV should be administered immediately after CAMPTOSAR, with the administration of 5-FU to occur immediately after receipt of LV. CAMPTOSAR should be used as recommended; the currently recommended regimens are shown in Table 10.

Table 10. Combination-Agent Dosage Regimens & Dose Modifications[a]

Regimen 1 6-wk cycle with bolus 5-FU/LV (next cycle begins on day 43)	CAMPTOSAR	125 mg/m^2 IV over 90 min, d 1,8,15,22		
	LV	20 mg/m^2 IV bolus, d 1,8,15,22		
	5-FU	500 mg/m^2 IV bolus, d 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		180 mg/m^2 IV over 90 min, d 1,15,29		
	LV		200 mg/m^2 IV over 2 h, d 1,2,15,16,29,30		
	5-FU	Bolus	400 mg/m^2 IV bolus, d 1,2,15,16,29,30		
	5-FU	Infusion[b]	600 mg/m^2 IV over 22 h, d 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. It is recommended that patients receive premedication with antiemetic agents. Prophylactic or therapeutic administration of atropine should be considered in patients experiencing cholinergic symptoms. See PRECAUTIONS, General.

Dose Modifications

Patients should be carefully monitored for toxicity and assessed prior to each treatment. Doses of CAMPTOSAR and 5-FU should be modified as necessary to accommodate individual patient tolerance to treatment. Based on the recommended dose-levels described in Table 10, Combination-Agent Dosage Regimens & Dose Modifications, subsequent doses should be adjusted as suggested in Table 11, Recommended Dose Modifications for Combination Schedules. All dose modifications should be based on the worst preceding toxicity. After the first treatment, patients with active diarrhea should return to pre-treatment bowel function without requiring anti-diarrhea medications for at least 24 hours before the next chemotherapy administration.

A new cycle of therapy should not begin until the toxicity has recovered to NCI grade 1 or less. Treatment maybe delayed 1 to 2 weeks to allow for recovery from treatment-related toxicity. If the patient has not recovered, consideration should be given to discontinuing therapy. Provided intolerable toxicity does not develop, treatment with additional cycles of CAMPTOSAR/5-FU/LV may be continued indefinitely as long as patients continue to experience clinical benefit.

Table 11. Recommended Dose Modifications for CAMPTOSAR/5-Fluorouracil (5-FU)/Leucovorin (LV) Combination Schedules

Patients should return to pre-treatment bowel function without requiring antidiarrhea medications for at least 24 hours before the next chemotherapy administration. A new cycle of therapy should not begin until the granulocyte count has recovered to ≥1500/mm^3, and the platelet count has recovered to ≥100,000/mm^3, and treatment-related diarrhea is fully resolved. Treatment should be delayed 1 to 2 weeks to allow for recovery from treatment-related toxicities. If the patient has not recovered after a 2-week delay, consideration should be given to discontinuing therapy

Toxicity NCI CTC Grade[a] (Value)	During a Cycle of Therapy	At the Start of Subsequent Cycles of Therapy[b]
No toxicity	Maintain dose level	Maintain dose level

Neutropenia		
1 (1500 to 1999/mm^3)	Maintain dose level	Maintain dose level
2 (1000 to 1499/mm^3)	↓ 1 dose level	Maintain dose level
3 (500 to 999/mm^3)	Omit dose until resolved to ≤ grade 2, then ↓ 1 dose level	↓ 1 dose level
4 (<500/mm^3)	Omit dose until resolved to ≤ grade 2, then ↓ 2 dose levels	↓ 2 dose levels
Neutropenic fever	Omit dose until resolved, then ↓ 2 dose levels	
Other hematologic toxicities	Dose modifications for leukopenia or thrombocytopenia during a cycle of therapy and at the start of subsequent cycles of therapy are also based on NCI toxicity criteria and are the same as recommended for neutropenia above.	
Diarrhea		
1 (2-3 stools/day > pretx[c])	Delay dose until resolved to baseline, then give same dose	Maintain dose level
2 (4-6 stools/day > pretx)	Omit dose until resolved to baseline, then ↓ 1 dose level	Maintain dose level
3 (7-9 stools/day > pretx)	Omit dose until resolved to baseline, then ↓ 1 dose level	↓ 1 dose level
4 (≥10 stools/day > pretx)	Omit dose until resolved to baseline, then ↓ 2 dose levels	↓ 2 dose levels
Other nonhematologic toxicities[d]		
1	Maintain dose level	Maintain dose level
2	Omit dose until resolved to ≤ grade 1, then ↓ 1 dose level	Maintain dose level
3	Omit dose until resolved to ≤ grade 2, then ↓ 1 dose level	↓ 1 dose level
4	Omit dose until resolved to ≤ grade 2, then ↓ 2 dose levels	↓ 2 dose levels
	For mucositis/stomatitis decrease only 5-FU, not CAMPTOSAR	*For mucositis/stomatitis decrease only 5-FU, not CAMPTOSAR.*

[a] National Cancer Institute Common Toxicity Criteria (version 1.0)
[b] Relative to the starting dose used in the previous cycle
[c] Pretreatment
[d] Excludes alopecia, anorexia, asthenia

Single-Agent Dosage Schedules

Dosage Regimens

CAMPTOSAR should be administered as an intravenous infusion over 90 minutes for both the weekly and once-every-3-week dosage schedules (see Preparation of Infusion Solution). Single-agent dosage regimens are shown in Table 12.

Table 12. Single-Agent Regimens of CAMPTOSAR and Dose Modifications

Weekly Regimen[a]	125 mg/m^2 IV over 90 min, d 1,8,15,22 then 2-wk rest		
	Starting Dose & Modified Dose Levels[c] (mg/m^2)		
	Starting Dose	Dose Level -1	Dose Level -2
	125	100	75
Once-Every-3-Week Regimen[b]	350 mg/m^2 IV over 90 min, once every 3 wks[c]		
	Starting Dose & Modified Dose Levels (mg/m^2)		
	Starting Dose	Dose Level -1	Dose Level -2
	350	300	250

[a]Subsequent doses may be adjusted as high as 150 mg/m^2 or to as low as 50 mg/m^2 in 25 to 50 mg/m^2 decrements depending upon individual patient tolerance.
[b]Subsequent doses may be adjusted as low as 200 mg/m^2 in 50 mg/m^2 decrements depending upon individual patient tolerance.
[c]Provided intolerable toxicity does not develop, treatment with additional cycles may be continued indefinitely as long as patients continue to experience clinical benefit.

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.

It is recommended that patients receive premedication with antiemetic agents.

Prophylactic or therapeutic administration of atropine should be considered in patients experiencing cholinergic symptoms. See PRECAUTIONS, General.

Dose Modifications

Patients should be carefully monitored for toxicity and doses of CAMPTOSAR should be modified as necessary to accommodate individual patient tolerance to treatment. Based on recommended dose-levels described in Table 12, Single-Agent Regimens of CAMPTOSAR and Dose Modifications, subsequent doses should be adjusted as suggested in Table 13, Recommended Dose Modifications for Single-Agent Schedules. All dose modifications should be based on the worst preceding toxicity.

A new cycle of therapy should not begin until the toxicity has recovered to NCI grade 1 or less. Treatment may be delayed 1 to 2 weeks to allow for recovery from treatment-related toxicity. If the patient has not recovered, consideration should be given to discontinuing this combination therapy. Provided intolerable toxicity does not develop, treatment with additional cycles of CAMPTOSAR may be continued indefinitely as long as patients continue to experience clinical benefit.

Table 13. Recommended Dose Modifications For Single-Agent Schedules[a]

A new cycle of therapy should not begin until the granulocyte count has recovered to ≥1500/mm^3, and the platelet count has recovered to ≥100,000/mm^3, and treatment-related diarrhea is fully resolved. Treatment should be delayed 1 to 2 weeks to allow for recovery from treatment-related toxicities. If the patient has not recovered after a 2-week delay, consideration should be given to discontinuing CAMPTOSAR.

Worst Toxicity NCI Grade[b] (Value)	**During a Cycle of Therapy**	**At the Start of the Next Cycles of Therapy (After Adequate Recovery), Compared with the Starting Dose in the Previous Cycle[a]**	
	Weekly	Weekly	Once Every 3 Weeks
No toxicity	Maintain dose level	↑ 25 mg/m^2 up to a maximum dose of 150 mg/m^2	Maintain dose level
Neutropenia			
1 (1500 to 1999/mm^3)	Maintain dose level	Maintain dose level	Maintain dose level
2 (1000 to 1499/mm^3)	↓ 25 mg/m^2	Maintain dose level	Maintain dose level
3 (500 to 999/mm^3)	Omit dose until resolved to ≤ grade 2, then ↓ 25 mg/m^2	↓ 25 mg/m^2	↓ 50 mg/m^2
4 (<500/mm^3)	Omit dose until resolved to ≤ grade 2, then ↓ 50 mg/m^2	↓ 50 mg/m^2	↓ 50 mg/m^2
Neutropenic fever	Omit dose until resolved, then ↓ 50 mg/m^2 when resolved	↓ 50 mg/m^2	↓ 50 mg/m^2
Other hematologic toxicities	Dose modifications for leukopenia, thrombocytopenia, and anemia during a cycle of therapy and at the start of subsequent cycles of therapy are also based on NCI toxicity criteria and are the same as recommended for neutropenia above.		
Diarrhea			
1 (2-3 stools/day > pretx[c])	Maintain dose level	Maintain dose level	Maintain dose level
2 (4-6 stools/day > pretx)	↓ 25 mg/m^2	Maintain dose level	Maintain dose level
3 (7-9 stools/day > pretx)	Omit dose until resolved to ≤ grade 2, then ↓ 25 mg/m^2	↓ 25 mg/m^2	↓ 50 mg/m^2
4 (≥10 stools/day > pretx)	Omit dose until resolved to ≤ grade 2 then ↓ 50 mg/m^2	↓ 50 mg/m^2	↓ 50 mg/m^2
Other nonhematologic[d] toxicities			
1	Maintain dose level	Maintain dose level	Maintain dose level
2	↓ 25 mg/m^2	↓ 25 mg/m^2	↓ 50 mg/m^2
3	Omit dose until resolved to ≤ grade 2, then ↓ 25 mg/m^2	↓ 25 mg/m^2	↓ 50 mg/m^2
4	Omit dose until resolved to ≤ grade 2, then ↓ 50 mg/m^2	↓ 50 mg/m^2	↓ 50 mg/m^2

[a] All dose modifications should be based on the worst preceding toxicity
[b] National Cancer Institute Common Toxicity Criteria (version 1.0)
[c] Pretreatment
[d] Excludes alopecia, anorexia, asthenia

Dosage in Patients with Reduced UGT1A1 Activity

When administered in combination with other agents, or as a single-agent, a reduction in the starting dose by at least one level of CAMPTOSAR should be considered for patients known to be homozygous for the UGT1A1*28 allele (see CLINICAL PHARMACOLOGY and WARNINGS). However, the precise dose reduction in this

patient population is not known and subsequent dose modifications should be considered based on individual patient tolerance to treatment (see Tables 10-13).

Preparation & Administration Precautions

As with other potentially toxic anticancer agents, care should be exercised in the handling and preparation of infusion solutions prepared from CAMPTOSAR Injection. The use of gloves is recommended. If a solution of CAMPTOSAR contacts the skin, wash the skin immediately and thoroughly with soap and water. If CAMPTOSAR contacts the mucous membranes, flush thoroughly with water.

Several published guidelines for handling and disposal of anticancer agents are available.[1-7]

Preparation of Infusion Solution

Inspect vial contents for particulate matter and repeat inspection when drug product is withdrawn from vial into syringe.

CAMPTOSAR Injection must be diluted prior to infusion. CAMPTOSAR should be diluted in 5% Dextrose Injection, USP, (preferred) or 0.9% Sodium Chloride Injection, USP, to a final concentration range of 0.12 to 2.8 mg/mL. In most clinical trials, CAMPTOSAR was administered in 250 mL to 500 mL of 5% Dextrose Injection, USP.

The solution is physically and chemically stable for up to 24 hours at room temperature (approximately 25°C) and in ambient fluorescent lighting. Solutions diluted in 5% Dextrose Injection, USP, and stored at refrigerated temperatures (approximately 2° to 8°C), and protected from light are physically and chemically stable for 48 hours. Refrigeration of admixtures using 0.9% Sodium Chloride Injection, USP, is not recommended due to a low and sporadic incidence of visible particulates. Freezing CAMPTOSAR and admixtures of CAMPTOSAR may result in precipitation of the drug and should be avoided. Because of possible microbial contamination during dilution, it is advisable to use the admixture prepared with 5%Dextrose Injection, USP, within 24 hours if refrigerated (2° to 8°C, 36° to 46°F). In the case of admixtures prepared with 5% Dextrose Injection, USP, or Sodium Chloride Injection, USP, the solutions should be used within 6 hours if kept at room temperature (15° to 30°C, 59° to 86°F).

Other drugs should not be added to the infusion solution. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

HOW SUPPLIED

Each mL of CAMPTOSAR Injection contains 20 mg irinotecan (on the basis of the trihydrate salt); 45 mg sorbitol; and 0.9 mg lactic acid. When necessary, pH has been adjusted to 3.5 (range, 3.0 to 3.8) with sodium hydroxide or hydrochloric acid.

CAMPTOSAR Injection is available in single-dose amber glass vials in the following package sizes:

2 mL	NDC 0009-7529-02
5 mL	NDC 0009-7529-01

The vial should be inspected for damage and visible signs of leaks before removing from the carton. If damaged, incinerate the unopened package.

Store at controlled room temperature 15° to 30°C (59° to 86°F). Protect from light. It is recommended that the vial should remain in the carton until the time of use.

Rx only

REFERENCES

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4. Polovich, M., White, J. M., & Kelleher, L.O. (eds.) 2005. Chemotherapy and biotherapy guidelines and recommendations for practice (2nd. ed.) Pittsburgh, PA: Oncology Nursing Society.

Camptosar brand of irinotecan hydrochloride injection

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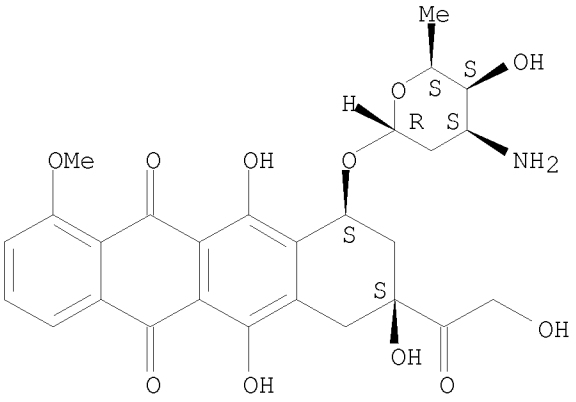
L1 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2010 ACS on STN
RN 23214-92-8 REGISTRY
ED Entered STN: 16 Nov 1984
CN 5,12-Naphthacenedione, 10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-
    hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(2-
    hydroxyacetyl)-1-methoxy-, (8S,10S)- (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN 5,12-Naphthacenedione, 10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-
    hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-
    1-methoxy-, (8S-cis)-
CN 5,12-Naphthacenedione, 10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-
    hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-
    1-methoxy-, (8S,10S)- (9CI)
OTHER NAMES:
CN 14-Hydroxydaunomycin
CN Biotransdox
CN Caelyx
CN Doxil
CN Doxorubicin
CN Evacet
CN Hydroxydaunomycin
CN Hydroxydaunorubicin
CN NSC 123127
CN PK 2
CN Rubex
FS STEREOSEARCH
DR 24385-08-8, 25311-50-6, 23257-17-2, 29042-30-6
MF C27 H29 N O11
CI COM
LC STN Files: ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUIRE, BEILSTEIN*,
      BIOSIS, BIOTECHNO, CA, CABA, CAPLUS, CASREACT, CBNB, CHEMCATS, CHEMLIST,
      CIN, CSCHEM, CSNB, DDFU, DRUGU, EMBASE, HSDB*, IFICDB, IFIPAT, IFIUDB,
      IMSDRUGNEWS, IMSPATENTS, IMSPRODUCT, IMSRESEARCH, IPA, MEDLINE, MRCK*,
      MSDS-OHS, NAPRALERT, PATDPASPC, PROMT, PROUSDDR, PS, RTECS*, SYNTHLINE,
      TOXCENTER, USAN, USPAT2, USPATFULL, USPATOLD, VETU
        (*File contains numerically searchable property data)
    Other Sources: DSL**, EINECS**, WHO
        (**Enter CHEMLIST File for up-to-date regulatory information)

Absolute stereochemistry.



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

22461 REFERENCES IN FILE CA (1907 TO DATE)

 1302 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
22646 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L2 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2010 ACS on STN
RN 97682-44-5 REGISTRY
ED Entered STN: 18 Aug 1985
CN [1,4'-Bipiperidine]-1'-carboxylic acid,
    (4S)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1H-
    pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl ester (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN 1H-Pyrano[3',4':6,7]indolizino[1,2-b]quinoline,
    [1,4'-bipiperidine]-1'-carboxylic acid deriv.
CN [1,4'-Bipiperidine]-1'-carboxylic acid,
    4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1H-
    pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl ester, (S)-
OTHER NAMES:
CN (+)-Irinotecan
CN 64: PN: WO2010011890 PAGE: 37 claimed sequence
CN Irinophore C
CN Irinotecan
CN Irinotecan lactone
FS STEREOSEARCH
MF C33 H38 N4 O6
CI COM
SR CA
LC STN Files: ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, BEILSTEIN*, BIOSIS,
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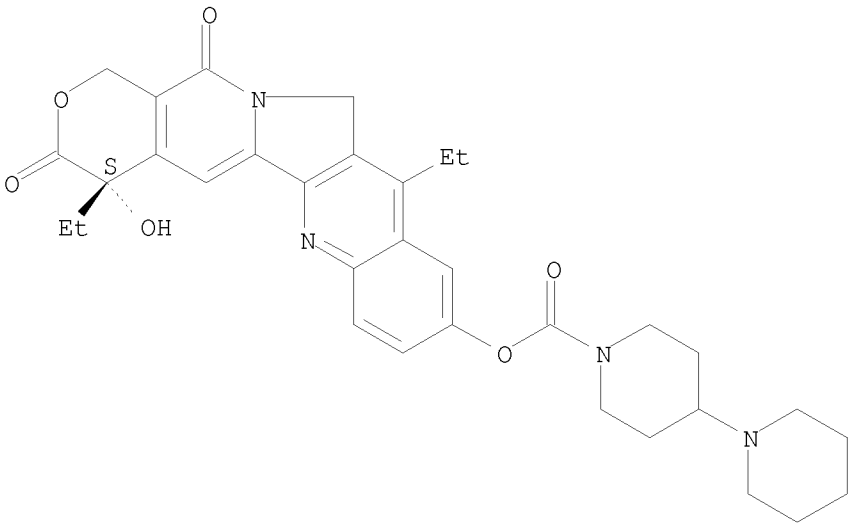

PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

            3823 REFERENCES IN FILE CA (1907 TO DATE)
              80 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
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Effect of Baseline Carbohydrate Antigen 19-9 (CA 19-9) Level on Overall Survival (OS) in NAPOLI-1 Trial: a Phase 3 Study of MM-398 (nal-IRI), with or without 5-Fluorouracil and Leucovorin (5-FU/LV), versus 5-FU/LV in Metastatic Pancreatic Cancer (mPAC) Previously Treated with Gemcitabine-based Therapy

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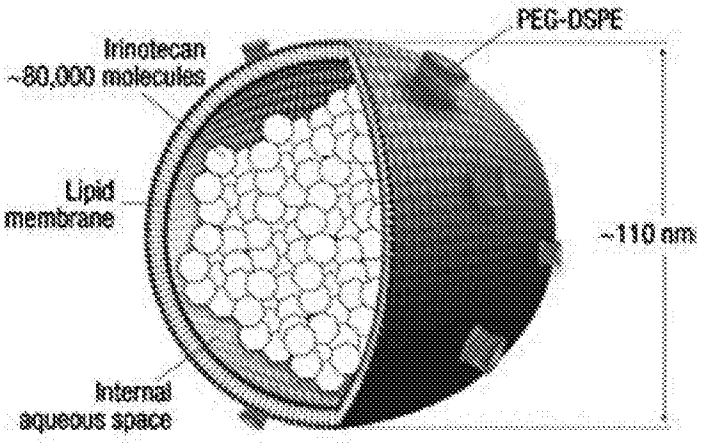
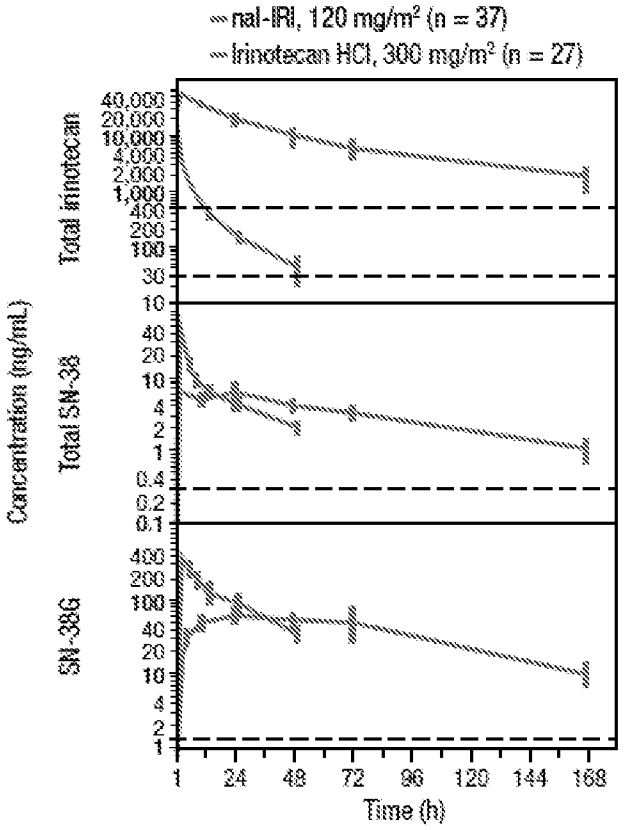
BACKGROUND

- Biomarkers for therapeutic response are needed to determine which patients may be most likely to respond to new and existing agents.
- CA19-9 levels have been shown to correlate positively with clinical stage and inversely with response to chemotherapy and survival in mPAC patients.[1-4]
  - CA19-9 may thus be useful in predicting response to therapy in patients with mPAC.
- nal-IRI (ONIVYDE™ [irinotecan liposome injection]; MM-398) is a nanoliposomal formulation of irinotecan, a topoisomerase inhibitor, for intravenous use (**Figure 1**[5]).
  - 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**).[6,7]
  - The liposome facilitates intratumoral drug deposition through the enhanced permeability and retention effect.[8]
  - 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**).[5]



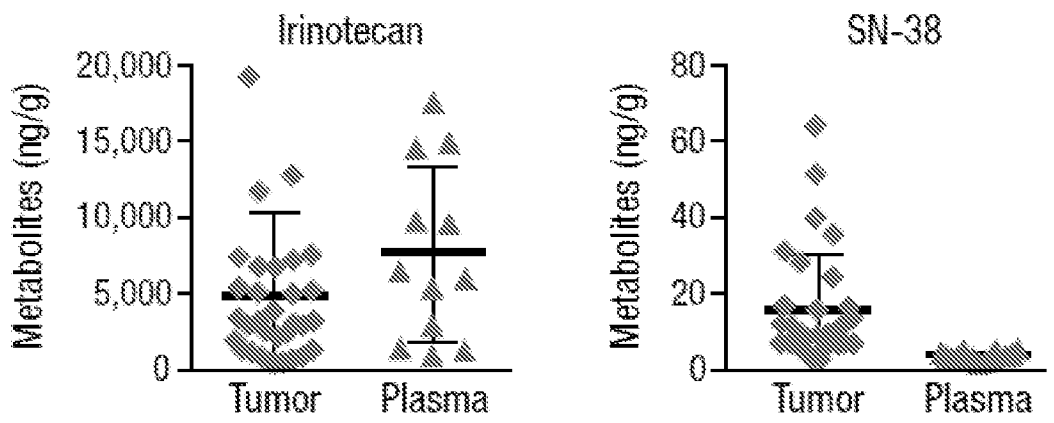
nal-IRI, nanoliposomal irinotecan.

Figure 1. nal-IRI design[5]



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[57]



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

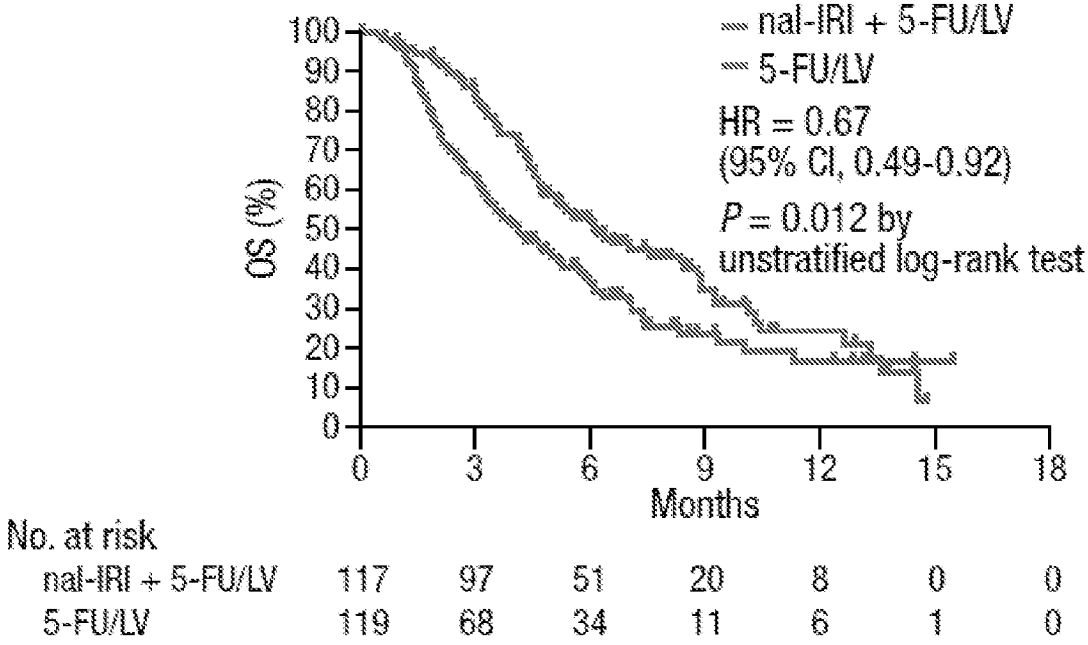
Drug metabolite quantification in tumor biopsies and plasma analyses from a study of patients (N = 14) with advanced solid tumors.

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[8]

- nal-IRI was recently approved by the US Food and Drug Administration 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), Phase 3 NAPOLI-1 trial of nal-IRI in this setting.[9]
  - 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**).



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[9]

- 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]; $P = 0.0001$) was also improved with nal-IRI + 5-FU/LV compared with 5-FU/LV.
- Objective response rate (ORR; complete or partial response) was 16% for patients in the nal-IRI + 5-FU/LV arm compared with 1% in the 5-FU/LV arm ($P < 0.0001$).

- Among patients with baseline CA19-9 >30 U/mL, CA19-9 response (≥50% decline from baseline level) was significantly higher with nal-IRI + 5-FU/LV than with 5-FU/LV alone (29% vs 9%; $P = 0.0006$).
- nal-IRI + 5-FU/LV demonstrated a predictable and manageable safety profile; the most frequently reported grade ≥3 treatment-emergent adverse events (TEAEs) were neutropenia (27%), fatigue (14%), diarrhea (13%), and vomiting (11%).

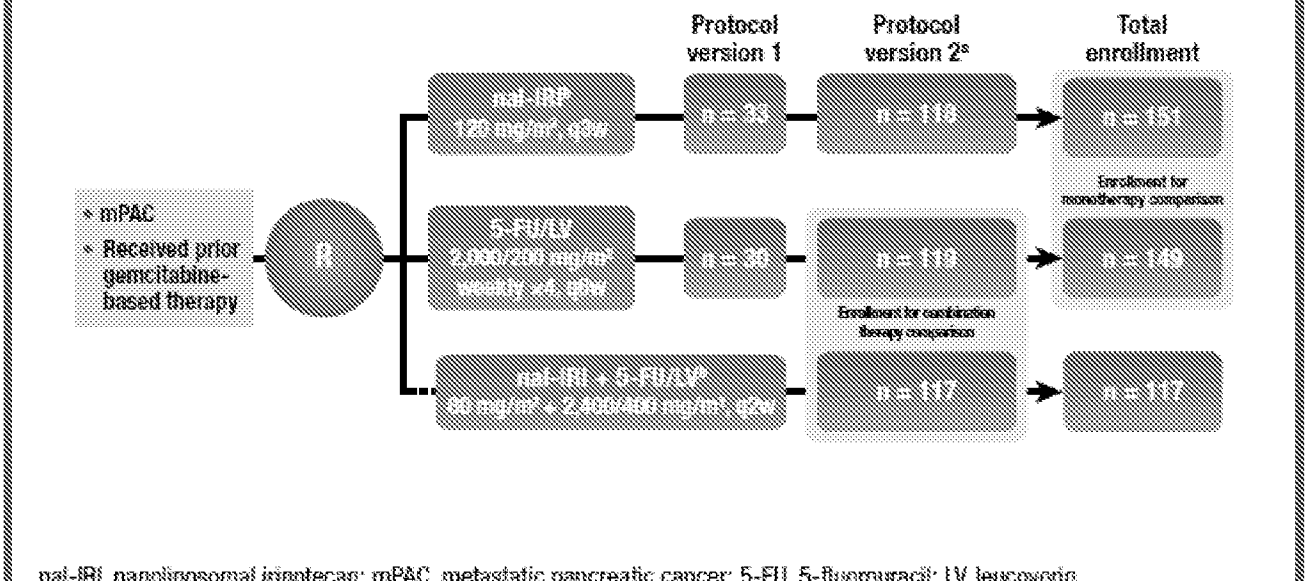
OBJECTIVE

- The objective of the current analysis of the NAPOLI-1 trial is to evaluate a potential predictive and/or prognostic effect between baseline CA19-9 levels and efficacy in patients receiving nal-IRI + 5-FU/LV versus 5-FU/LV alone.

NAPOLI-1 METHODS

Study Design

- NAPOLI-1 was an international, randomized, open-label, Phase 3 trial (**Figure 5**).
  - Patients were initially randomized to nal-IRI monotherapy or 5-FU/LV.
  - The protocol was amended to add a third arm of the combination of nal-IRI + 5-FU/LV once safety data of the combination became available from a concurrent study in metastatic colorectal cancer. The decision to add the third arm was made shortly after the trial was initiated, and 63 patients were enrolled under protocol version 1 before all sites switched to protocol version 2. Combination therapy was compared with only those patients in the 5-FU/LV control arm who were randomized after the protocol amendment.
  - Randomization was stratified by baseline albumin levels, Karnofsky performance status (KPS), and ethnicity.



nal-IRI, nanoliposomal irinotecan; mPAC, metastatic pancreatic cancer; 5-FU, 5-fluorouracil; LV, leucovorin.
[a]NAPOLI-1 was amended to add the nal-IRI + 5-FU/LV arm once safety data on the combination became available. Only those patients enrolled in the 5-FU/LV arm after the amendment (n = 119) were used as the control for the combination arm.
[b]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 nal-IRI doses of 120 and 80 mg/m^2 approximate to 100 and 70 mg/m^2, respectively, based on irinotecan free base.

Figure 5. NAPOLI-1 study design

Key Inclusion Criteria

- Metastatic pancreatic ductal adenocarcinoma (measurable or non-measurable)
- Disease progression after prior gemcitabine or gemcitabine-containing therapy in a neoadjuvant, adjuvant (only if distant metastases occurred within 6 months of completing adjuvant therapy), locally advanced, or metastatic setting
- KPS ≥70
- Adequate hematologic (including absolute neutrophil count >1,500/μL), hepatic (including normal serum total bilirubin and albumin levels ≥3.0 g/dL), and renal function
- Adults ≥18 years of age

CA19-9 Assessment

- Blood samples were taken at baseline and every 6 weeks thereafter until disease progression, initiation of a new antineoplastic treatment, or withdrawal of consent and assessed for CA19-9 levels by a central laboratory in order to evaluate the predictive and prognostic roles of CA19-9.

CA19-9 Quartile Analysis

- The analysis of outcomes by baseline CA19-9 levels was not a pre-specified analysis.
- For this analysis, the results of baseline CA19-9 measurements were divided into quartiles (Q1-Q4).
- Treatment comparisons were carried out by an unstratified Cox proportional hazards regression model to estimate HRs and corresponding 95% CIs for the effect of baseline CA19-9 levels on OS, PFS, and ORR.

RESULTS

Patient Characteristics

- A total of 76 sites in 14 countries enrolled 417 patients between January 2012 and September 2013.
- Patient demographic and baseline clinical characteristics were well balanced between the nal-IRI + 5-FU/LV arm (n = 117) and the 5-FU/LV control arm (n = 119; **Table 1**).

Table 1. Patient Demographic and Baseline Clinical Characteristics

Parameter	nal-IRI + 5-FU/LV (n = 117)	5-FU/LV (n = 119)
Median age (IQR), y	63 (57-70)	62 (55-69)
KPS, %		
100	15	14
90	44	34
80	32	43
70	6	8
50-60	3	0
Race, %		
Caucasian	62	64
East Asian	29	30
Other	9	6
CA19-9 ≥40 U/mL, %[a]	81	80
Pancreatic head tumor, %	65	58
Prior lines of metastatic therapy, %		
0[b]	13	13
1	53	56
2	34	31

nal-IRI, nanoliposomal irinotecan; 5-FU, 5-fluorouracil; LV, leucovorin; IQR, interquartile range; KPS, Karnofsky performance status; CA19-9, carbohydrate antigen 19-9.

[a]Includes only patients who had a measured CA19-9 value prior to treatment. Data were missing for 3 patients in the nal-IRI + 5-FU/LV group and 5 patients in the 5-FU/LV group.

[b]Patients received neoadjuvant, adjuvant, or locally advanced treatment, but had no previous therapy for metastatic disease.

- Quartile ranges were based on 404 available baseline CA19-9 values from the 417 randomized patients in NAPOLI-1.
- Of the 236 patients randomized to receive nal-IRI + 5-FU/LV or 5-FU/LV alone (after the protocol amendment), 218 received study treatment and had a baseline CA19-9 measurement, and were included in this analysis (**Table 2**).

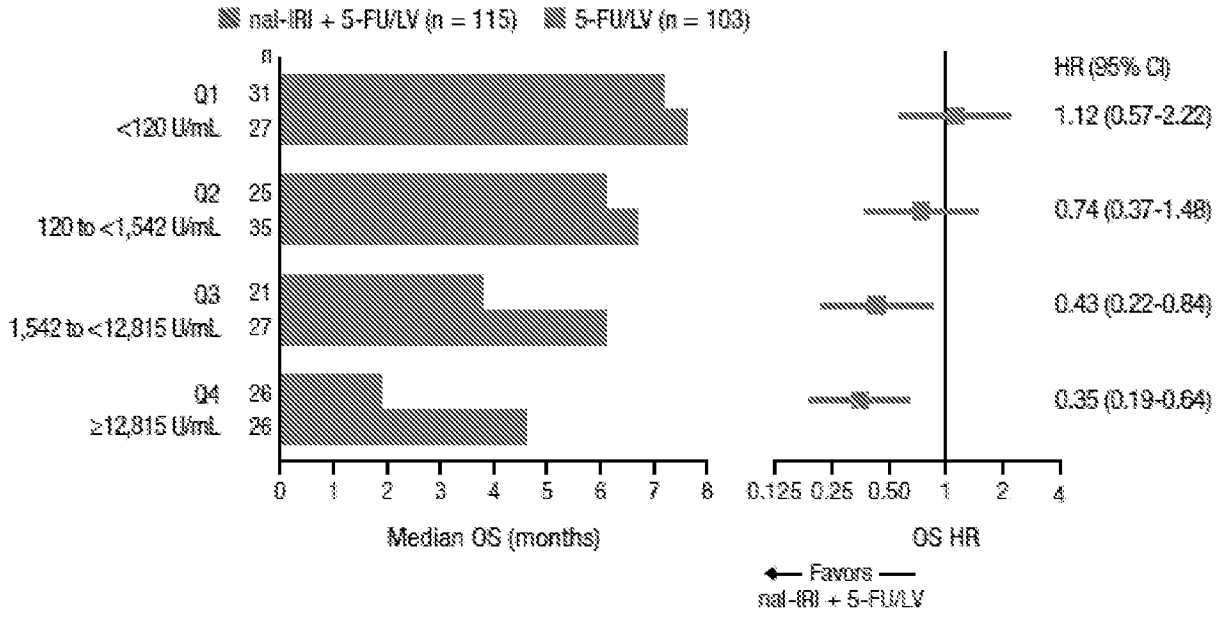
Table 2. CA19-9 Quartiles

	CA19-9 level (U/mL)	nal-IRI + 5-FU/LV, n	5-FU/LV, n
Quartile 1	<120	27	31
Quartile 2	120 to <1,542	35	25
Quartile 3	1,542 to <12,815	27	21
Quartile 4	≥12,815	26	26

CA19-9, carbohydrate antigen 19-9; nal-IRI, nanoliposomal irinotecan; 5-FU, 5-fluorouracil; LV, leucovorin.

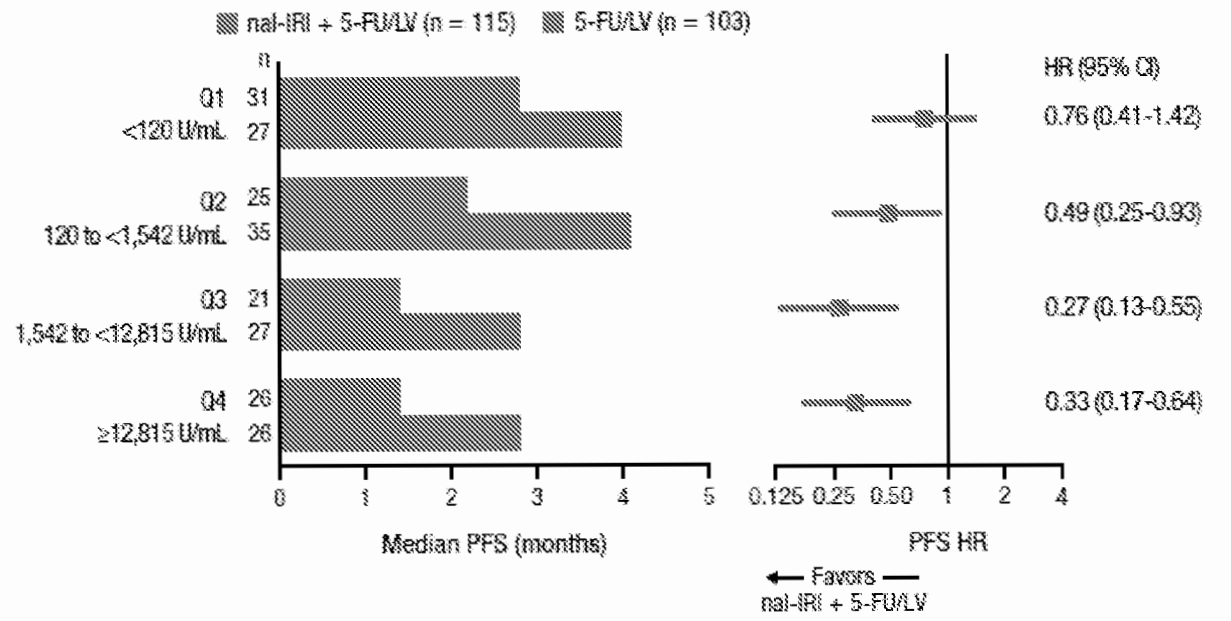
Association of Baseline CA19-9 Levels with Efficacy

- A greater treatment effect of nal-IRI + 5-FU/LV on OS and PFS relative to 5-FU/LV was observed with higher baseline CA19-9 levels (**Figure 6** and **Figure 7**).
- Stratified Kaplan-Meier OS and PFS curves are shown for each baseline CA19-9 quartile in **Figure 8** and **Figure 9**, respectively.



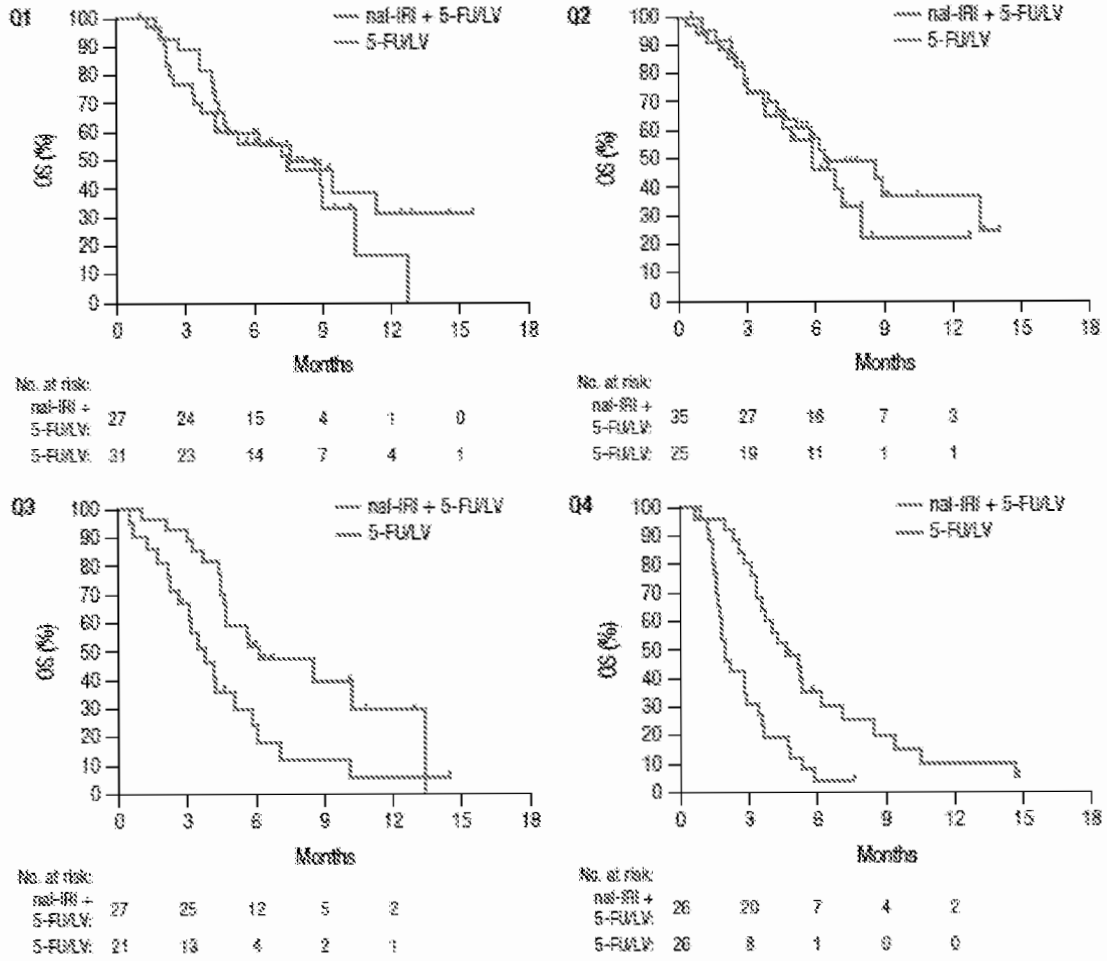
CA19-9, carbohydrate antigen 19-9; OS, overall survival; nal-IRI, nanoliposomal irinotecan; 5-FU, 5-fluorouracil; LV, leucovorin; HR, hazard ratio; CI, confidence interval.
HRs are shown with their 95% CIs.

Figure 6. Association of baseline CA19-9 levels with OS



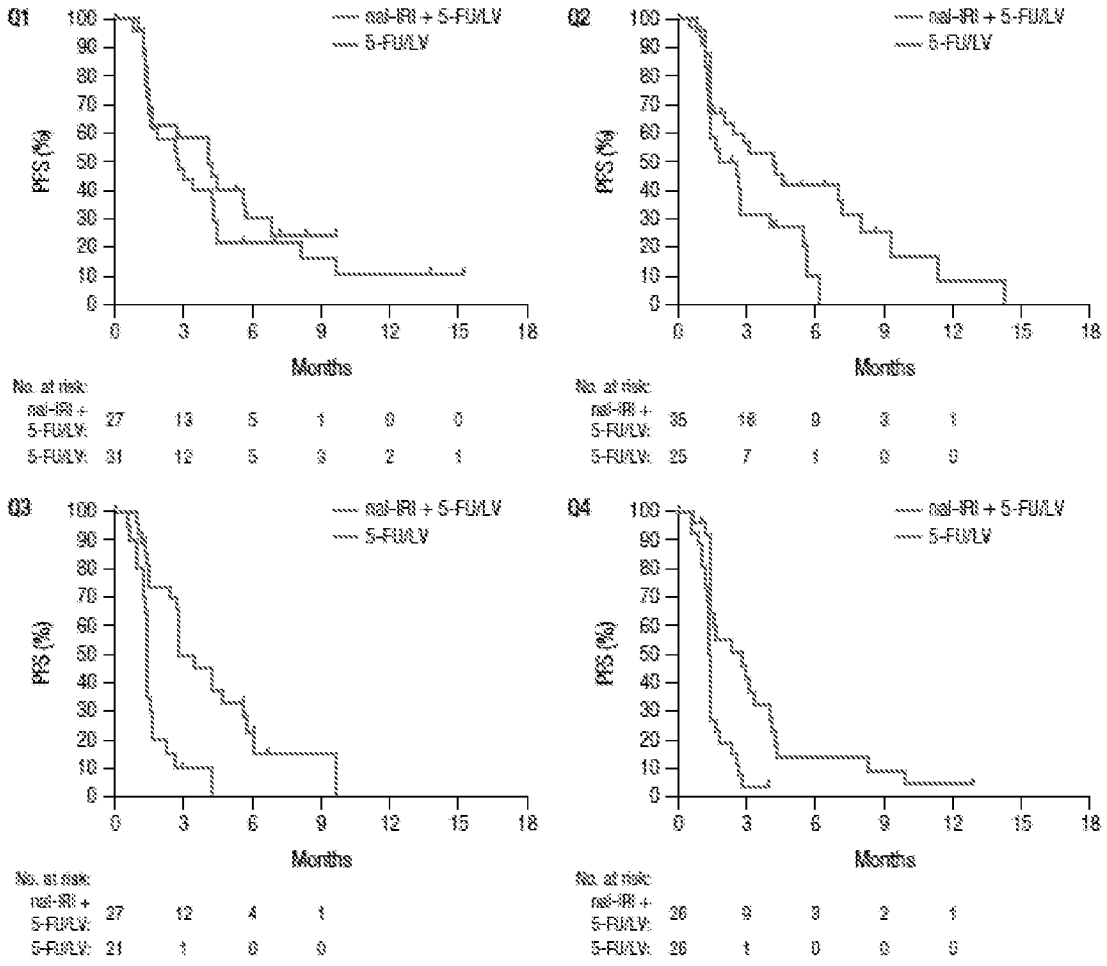
CA19-9, carbohydrate antigen 19-9; PFS, progression-free survival; nal-IRI, nanoliposomal irinotecan; 5-FU, 5-fluorouracil; LV, leucovorin; HR, hazard ratio; CI, confidence interval.
HRs are shown with their 95% CIs.

Figure 7. Association of baseline CA19-9 levels with PFS



OS, overall survival; CA19-9, carbohydrate antigen 19-9; nal-IRI, nanoliposomal irinotecan; 5-FU, 5-fluorouracil; LV, leucovorin.

Figure 8. Kaplan-Meier OS by CA19-9 quartile



PFS, progression-free survival; CA19-9, carbohydrate antigen 19-9; nal-IRI, nanoliposomal irinotecan; 5-FU, 5-fluorouracil; LV, leucovorin.

Figure 9. Kaplan-Meier PFS by CA19-9 quartile

- ORR was higher in the nal-IRI + 5-FU/LV arm compared with the 5-FU/LV arm across all quartiles of baseline CA19-9 levels (**Table 3**).

Table 3. Association of Baseline CA19-9 Levels with ORR

	CA19-9 (U/mL) quartile			
ORR , n/N (%)	Q1 CA19-9 <120	Q2 120 ≤CA19-9 <1,542	Q3 1,542 ≤CA19-9 <12,815	Q4 CA19-9 ≥12,815
nal-IRI + 5-FU/LV (n = 115)	2/27 (7)	7/35 (20)	7/27 (26)	3/26 (12)
5-FU/LV (n = 103)	0/31 (0)	1/25 (4)	0/21 (0)	0/26 (0)

CA19-9, carbohydrate antigen 19-9; ORR, objective response rate; nal-IRI, nanoliposomal irinotecan; 5-FU, 5-fluorouracil; LV, leucovorin.

- In both treatment groups, there was a trend toward higher rates of treatment termination with increasing baseline CA19-9 quartile; however, there was no pattern with regard to the proportion of patients who went on to receive subsequent anti-cancer therapy (**Table 4**).

Table 4. Post-Treatment Anti-Cancer Therapy

Treatment	CA19-9 quartile	n	Terminated treatment, n (%)	Received post-treatment therapy, n (% of terminated)
nal-IRI + 5-FU/LV	1	27	22 (81)	5 (23)
	2	35	30 (86)	14 (47)
	3	27	24 (89)	11 (46)
	4	26	25 (96)	6 (24)
5-FU/LV	1	31	28 (90)	12 (43)
	2	25	23 (92)	13 (57)
	3	21	21 (100)	9 (43)
	4	26	26 (100)	8 (31)

CA19-9, carbohydrate antigen 19-9; nal-IRI, nanoliposomal irinotecan; 5-FU, 5-fluorouracil; LV, leucovorin.

CONCLUSIONS

- nal-IRI + 5-FU/LV significantly improved OS and PFS compared with 5-FU/LV control in the NAPOLI-1 trial of patients with mPAC following gemcitabine-based therapy.[9]
- Results of the current analysis confirmed data from prior reports[1,3,4] that baseline CA19-9 level is inversely associated with survival in patients with mPAC.
- The observed OS and PFS benefits for nal-IRI + 5-FU/LV over 5-FU/LV were greatest among patients with the highest baseline CA19-9 levels.
- ORR was greater with nal-IRI + 5-FU/LV relative to 5-FU/LV control in the overall population, and there was no clear trend in impact on ORR relative to baseline CA19-9.
- These results suggest that baseline CA19-9 levels are associated with the treatment effect observed for the combination of nal-IRI + 5-FU/LV in patients with mPAC.

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Abstract 234

Expanded analyses of NAPOLI-1: Phase 3 study of MM-398 (nal-IRI), with or without 5-fluorouracil and leucovorin, versus 5-fluorouracil and leucovorin, in metastatic pancreatic cancer (mPAC) previously treated with gemcitabine-based therapy

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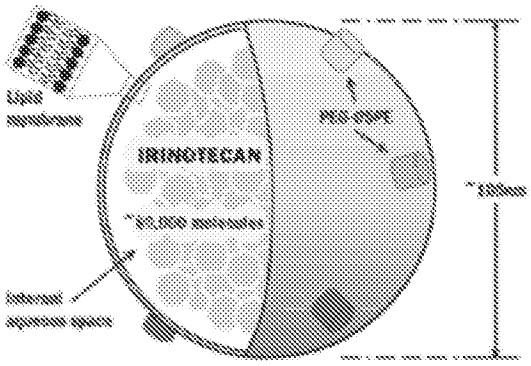
BACKGROUND

- Pancreatic cancer is the 4th leading cause of death in the United States and EU.[1,2]
- Currently there is no standard of care for patients with metastatic pancreatic cancer who have been previously treated with a gemcitabine containing regimen.
- Despite signs of activity in Phase 2 trials in metastatic pancreatic cancer, most drugs fail to demonstrate positive Phase 3 outcomes.
- Approximately 80% of patients with metastatic pancreatic cancer succumb to disease within 12 months.[3]
- NAPOLI-1 is an international randomized Phase 3 trial in metastatic pancreatic cancer patients previously treated with a gemcitabine based therapy.

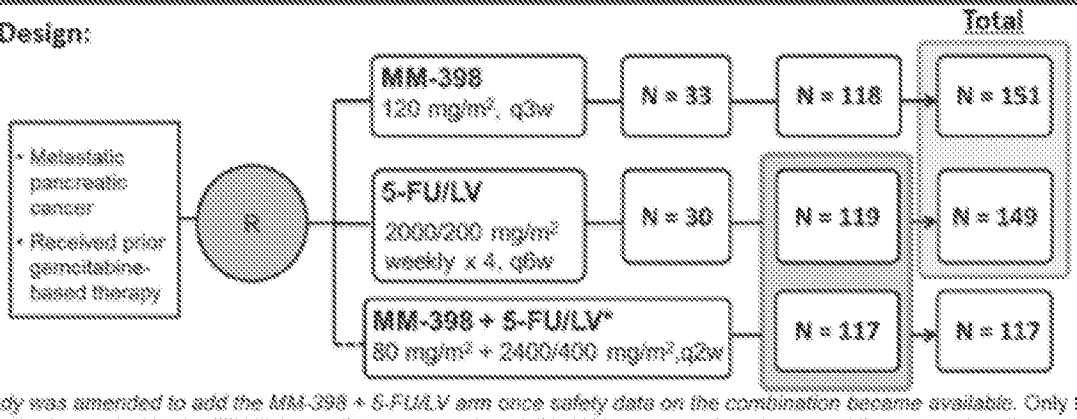
MM-398 (Liposome Irinotecan Injection)

- Contains ~80,000 molecules of irinotecan stably encapsulated in an ~100nm liposome
- MM-398 (120 mg/m²) has extended circulation
  - AUC of total irinotecan in blood is 1652 vs. 24 hr·µg/ml of conventional irinotecan (300 mg/m²) [4]
- MM-398 achieved 9.6 ng/g of SN-38 (active metabolite) in tumor compared to 1.7 ng/ml in blood at 72 hours [5]
- Median OS of 5.2 months for MM-398 in Phase 2 study in refractory metastatic pancreatic cancer [6]



NAPOLI-1 METHODS

Study Design:



* *Study was amended to add the MM-398 + 5-FU/LV arm once safety data on the combination became available. Only those patients enrolled in the 5FU/LV arm after the amendment (N=119), were used as the control for the combination arm.*

Study Objectives:

Primary: Overall Survival (OS)

Secondary: Progression Free Survival (PFS), Objective Response Rate (ORR), Tumor Marker Response (CA19-9) and Safety

Methodology:

- Open label, randomized, stratified by albumin (<4.0 g/dL vs ≥4.0 g/dL), KPS (70 & 80 vs ≥90), and ethnicity (Caucasian vs East Asian vs others)
- The primary analysis compared each treatment arm 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 98% power to detect HR=0.50 in the MM-398 + 5-FU/LV arm
- A supportive stratified analysis, accounting for the randomization strata, was also performed
- Data presented in this poster is from a protocol-defined primary analysis data cut, which took place on 14Feb2014, after 305 events

Key Inclusion Criteria:

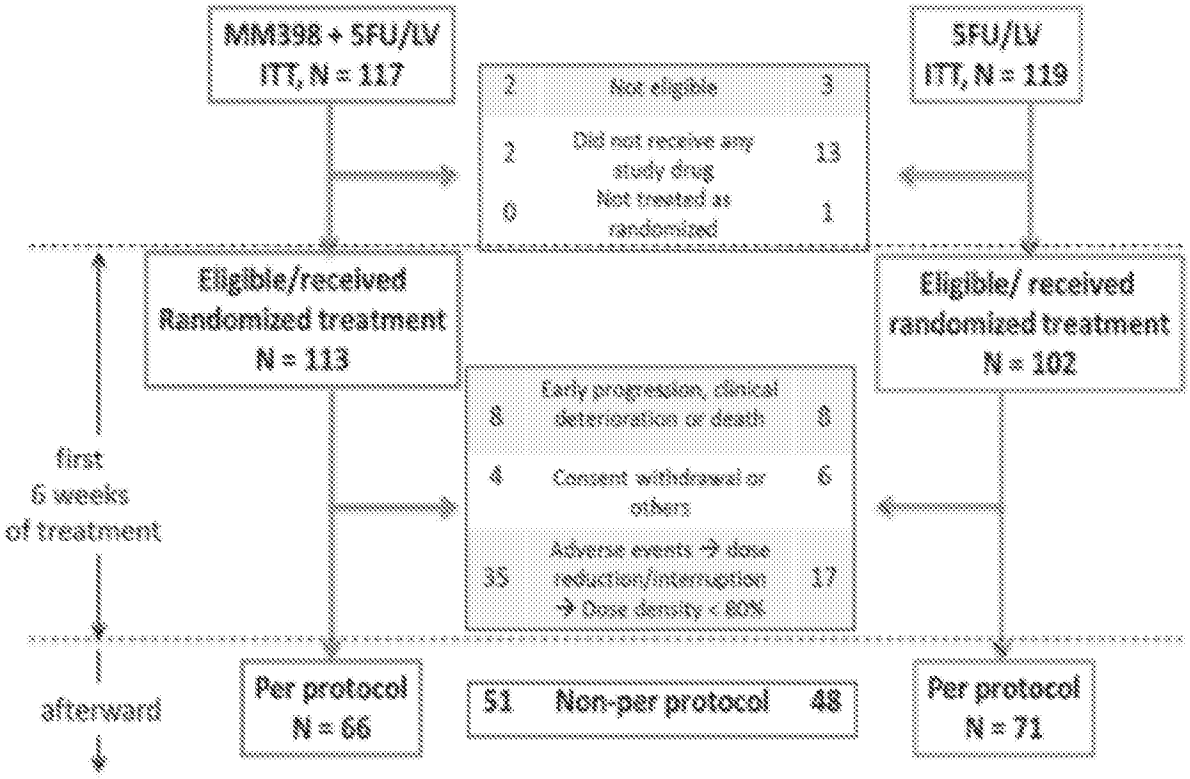
- Adenocarcinoma of the exocrine pancreas
- Metastatic disease, measurable or non-measurable
- Progressed after prior gemcitabine or gemcitabine-containing therapy
- KPS ≥70
- Adequate bone marrow, hepatic (bilirubin within normal range for the institution and albumin ≥3 g/dL), and renal function

EXPLANATION OF ITT AND PP POPULATIONS

ITT: All randomized patients

PP: Patients who received ≥80% of the protocol defined treatment during the first 6 weeks of treatment and did not have the following protocol violations:

* Receipt of any prohibited therapies as defined in the protocol
* Not receiving treatment as randomized
* Inclusion/exclusion criteria deviations



Patient Characteristics

- The NAPOLI-1 study was well balanced. Patients in the MM-398 + 5FU/LV and 5FU/LV arms were consistent across the following patient characteristics: prognostic factors, demographics (age, sex, race), tumor and pre and post treatment characteristics.
- Post-study anticancer therapy was 31% in the MM-398+5-FU/LV arm and 38% in the 5-FU/LV arm

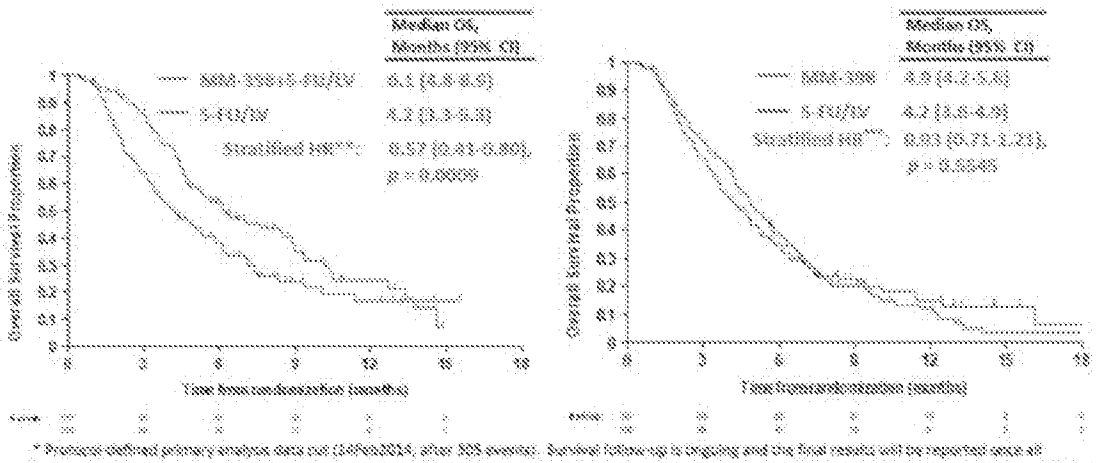
DEMOGRAPHIC CHARACTERISTICS: PP VS NON-PP

Parameter	MM-398 + 5FU/LV		5FU/LV	
	PP (N=66)	Non PP* (N=41)	PP (N=74)	Non PP (N=46)
KPS 90 and 100, %	62	49	61	50
Albumin ≥ 4.0 g/dL, %	48	41	46	42
Race, (%)				
Caucasian	71	56	63	63
East Asian	21	39	31	28
CA 19-9 ≥ 40, %*	82	78	76	86
Pancreatic head tumor, %	61	71	68**	44**
Liver Metastasis, %	64	65	75	65
Line of Treatment, %				
First line	14	12	13	13
Second line	53	53	59	52
Post-second line	33	35	28	35
Time since last therapy, months***	1.4 (0.9 , 2.1)	1.4 (1.0, 2.8)	1.2 (1.0, 2.3)	1.2 (1.0, 2.1)
Time since diagnosis, months***	10.3 (5.2, 15.8)	10.8 (6.6, 19.1)	10.3 (6.5, 15.1)	10.5 (5.6, 16.2)
Stage 4 at diagnosis, %	53	51	51	54

*Includes only patients who had a measured CA 19-9 prior to treatment; **Showed a statistically significant difference (p ≤ 0.01);
***Median (1st quartile, 3rd quartile)

- A total of 76 sites, of the 105 initiated, in 14 countries enrolled 417 patients between Jan 2012 and Sep 2013

Overall Survival: ITT*



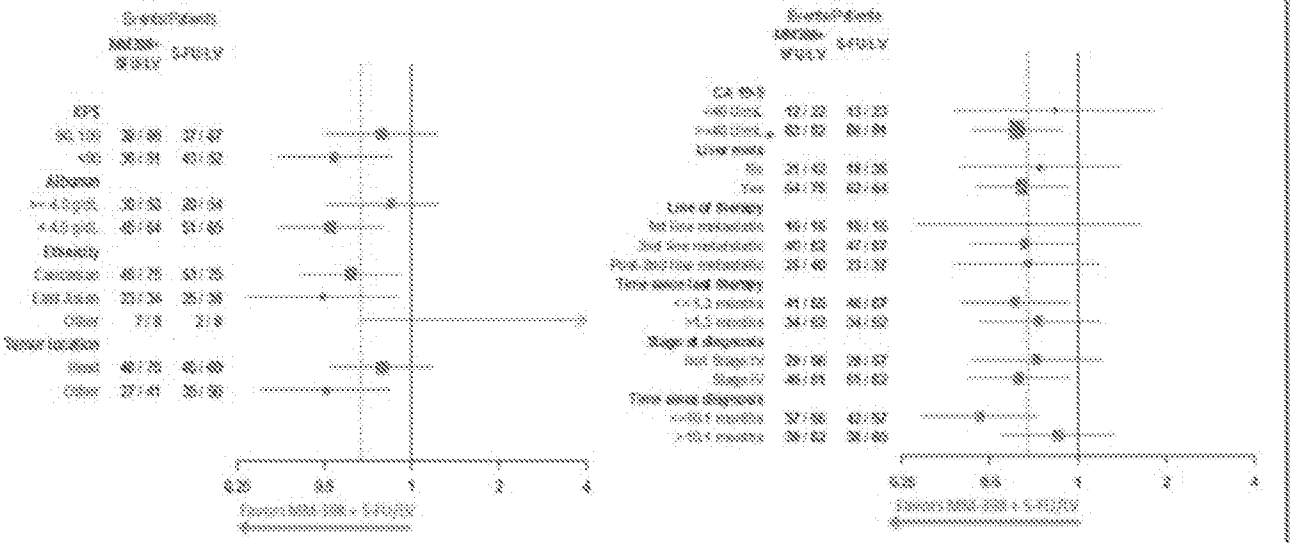
* Protocol-defined primary analysis data cut (14/Feb/2014), after 323 events. Survival follow-up is ongoing and the final results will be reported once all patients are off treatment and at least 80% events have taken place. ***Stratified HR: 0.67 (0.49-0.92), p = 0.0122; ****Unstratified HR: 0.68 (0.77-1.28), p = 0.8436

Tumor Response and Control

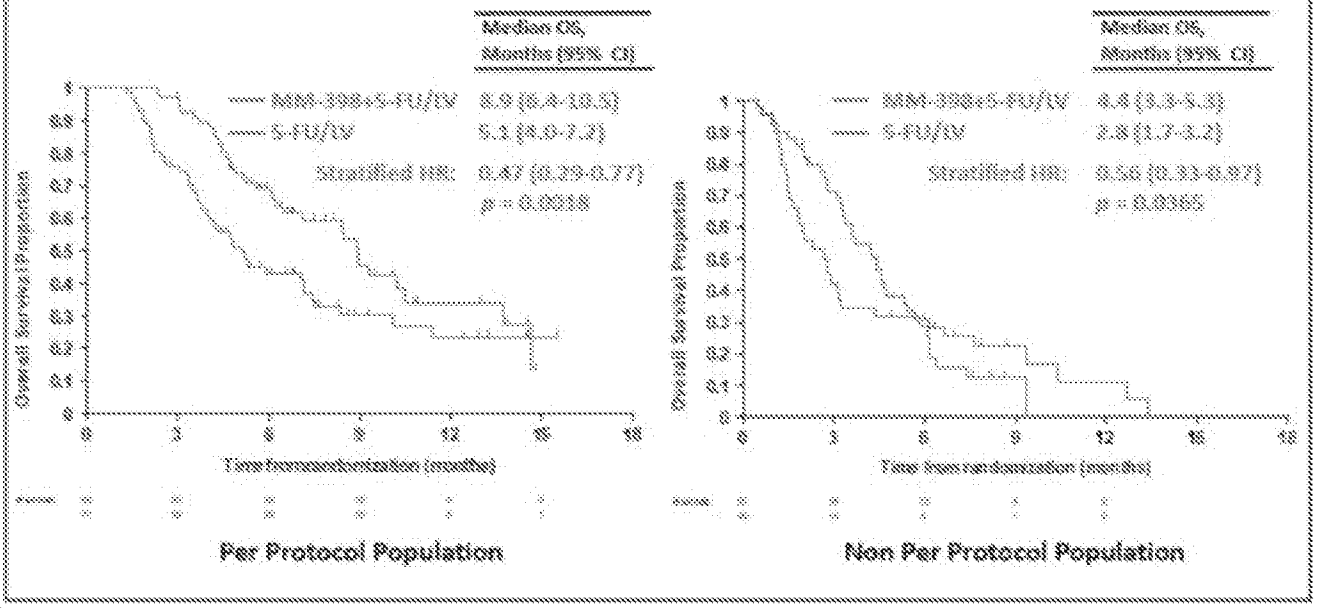
	MM-398+5-FU/LV (N=117)	5-FU/LV (N=119)	*p* Value
Median PFS, months (95% CI)	3.1 (2.7 – 4.2)	1.5 (1.4 – 1.8)	0.0001 (Log-rank test)
PFS rate at 12 weeks, % (95% CI)	57 (47 - 66)	26 (18 - 35)	-
Overall Response Rate, % * (95% CI)	16 (9.6 - 22.9)	1 (0.0 - 2.8)	< 0.001 (Fisher's exact test)
CA19-9 reduction, % ** (responders/evaluable pts.)	36 (27 / 76)	12 (8 / 69)	0.0009 (Fisher's exact test)

* Per RECIST version 1.1; **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

Sensitivity Analysis



Overall Survival: PP and Non-PP



SAFETY, DOSE MODIFICATIONS AND TREATMENT EXPOSURE

	Safety Population[a]		PP	
	MM-398 + 5-FU/LV (n=117)	5-FU/LV (n=134)	MM-398 + 5-FU/LV (n=66)	5-FU/LV (n=71)
Grade ≥3 nonhematologic AEs in >5% patients, %[b]				
Fatigue	14	4	14	6
Diarrhea	13	5	12	7
Vomiting	11	3	8	3
Nausea	8	3	9	1
Asthenia	8	7	5	6
Abdominal pain	7	6	5	3
Grade ≥3 hematologic AEs based on laboratory values, %[b,c]				
Neutrophil count decreased	20	2	15	3
Hemoglobin decreased	6	5	6	4
Platelet count decreased	2	0	2	0
Patients with at least 1 AE leading to death (all causes), %	2	7	0	6
Number of patients with Treatment Emergent Adverse Events resulting in, n (%)				
Dose Reduction	39 (33)	5 (4)	22 (33)	2 (3)
Dose Delays	72 (62)	43 (32)	40 (61)	15 (21)
Treatment Discontinuation	13 (11)	10 (8)	3 (5)	2 (3)
Average relative dose intensity (%)				
MM-398	83.2	-	85.4	-
5-FU	83.9	95.6	86.4	97.9
Average duration of exposure, weeks[d]	15	10	21	13

[a] Patients receiving at least one dose of study drug; [b] Per CTCAE Version 4; [c] Includes only patients who had at least one post-baseline assessment; [d] Duration of exposure is the time from (the date of the last administration of study drug + projected days to next dose of study drug administration - date of first study drug administration)/7

SUMMARY

- ITT analysis demonstrates statistically significant increase in overall survival (OS) of MM-398 + 5-FU/LV (MM-398 80 mg/m^2 q2w regimen) over 5-FU/LV
  - Significant increase also observed in PFS, ORR and CA19-9 response
  - MM-398 single agent, 120 mg/m^2 q3w regimen, did not show a significant difference in OS
- Sensitivity analyses favor MM-398 + 5-FU/LV over 5-FU/LV OS across prognostic subgroups, tumor characteristics, and previous treatment

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- Per Protocol analysis demonstrates that OS difference is maintained with MM-398 + 5-FU/LV combination regimen
  - Despite additional dose modifications, patients receiving MM-398+5-FU/LV were able to stay on treatment longer
- Safety profile was manageable, with most frequent grade ≥3 AEs including neutropenia, fatigue and GI effects (diarrhea and vomiting)

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Effect of Composition on the Stability of Liposomal Irinotecan Prepared by a pH Gradient Method

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Liposomal irinotecan was prepared by pH gradient loading. The parameters that govern this process, including drug loading time, incubation temperature, buffer composition for hydration, and ΔpH, were studied. The uptake of irinotecan into liposomal systems in response to the magnitude of the pH gradient was examined. The drug uptake was maximum when the magnitude of ΔpH approached 3.7. The effect of the formulation of the liposomes on the stability of the drug delivery system was also studied. Liposomes composed of lipids with a high phase transition temperature, L-α-distearoyl-phophatidyl-choline and hydrogenated soy phosphatidylcholine, were more stable than those composed of lipids with lower phase transition temperatures. Incorporating distearoyl phosphatidylethanolamine-poly (ethylene glycol)$_{2000}$ into liposomes helped to reduce the size of the liposomes. In addition, the retention of a drug within liposomes was found to be slightly enhanced by including dimyristoylphosphatidylglycerol or dextran sulfate in the liposome formulation.

[**Key words:** irinotecan, liposome, pH gradient, dimyristoylphosphatidylglycerol (DMPG), dextran sulfate]

Irinotecan (7-ethyl-10-(4-[1-piperidino]-1-piperidino) carboxylcamptothecin, CPT-11) is a water-soluble prodrug that can be converted to SN-38, an active metabolite that exhibits antitumor activity via the inhibition of topoisomerase I activity (1). This camptothecin-based drug has passed clinical trials and is currently approved for the treatment of colonic, ovarian, and small cell lung cancer, and is increasingly combined with other standard chemotherapeutic agents for enhanced therapy (2–4). However, irinotecan was discovered to have serious side effects such as myelosuppression and gastrointestinal disorders (mainly diarrhea), which are recognized as constituting dose-limiting toxicity for this drug (5, 6). The basic labile characteristic of the lactone E ring in irinotecan is reversible and pH-dependent hydrolysis yields the inactive carboxylate species (7). Only lactone species can inhibit topoisomerase I, but this active molecule is very rarely found under physiological conditions (8). Consequently, finding an effective drug delivery system to reduce toxicity and preserve the active form of the drug is very important.

Liposomes have been extensively used as drug carriers (9). Recent reports have asserted that the liposomal formulation of irinotecan, which includes polyethyleneglycol, can reduce the clearance of liposomes *in vivo* and increase antitumor activity (10, 11). Nevertheless, very few published reports have investigated in depth the preparation and stability of liposomal irinotecan.

This study addresses the influence of the composition of liposomes and the experimental preparation parameters on the delivery system to elucidate the stability of liposomes that contain irinotecan. Furthermore, a variety of amino-containing drugs can be encapsulated into liposomes in response to transmembrane pH gradients (12, 13). The uptake of irinotecan by intestinal epithelial cells has been demonstrated to depend on pH (14). Consequently, a pH-gradient method was used here to prepare liposomal irinotecan and the effect of pH on the loading of drugs was examined.

Before the preparation of liposomes, irinotecan solution (20 mg/ml), egg phosphatidylcholine (EPC), L-α-distearoyl-phophatidyl-choline (DSPC), distearoyl phosphatidylethanolamine-poly (ethylene glycol)$_{2000}$ (DSPE-PEG$_{2000}$), hydrogenated soy phosphatidylcholine (HSPC), dimyristoylphosphatidylglycerol (DMPG), cholesterol, and dextran sulfate were purchased. Then, dry lipids were mixed in the desired molar ratio and dissolved in the $CHCl_3$/methanol (v/v=9/1) cosolvent. A lipid thin film was formed by removing solvent under a high vacuum at 60°C. Lipid films were hydrated by adding 2 ml of citrate buffer vortexed extensively for 20 min, and then sonicated at a controlled temperature, according to the formula used. A liposome solution with a lipid concentration of 5 mM was attained. However, in the case of liposomes that contained dextran sulfate, the desired concentration of dextran sulfate was added to the citrate buffer prior to hydration. Finally, liposome suspensions were passed through a 0.22-μm sterile filter to remove larger particles and obtain a desirable size distribution. The size distribution of liposomes was determined by dynamic light scattering (LPA3000/3100 particle analyzer; Otsuka Electronics, Osaka). All liposomes prepared in this study had average diameters in the range between 80 to 140 nm.

Irinotecan was loaded into liposomes in response to a

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TABLE 1. Uptake ratio of irinotecan into liposomes of EPC/cholesterol (EPC/Chol) or EPC/cholesterol/DSPE-PEG_{2000} (EPC/Chol/DSPE-PEG_{2000}) with different pH gradients at 40°C

Lipid	pH	Uptake %	P.S. (nm)
EPC/Chol	1.93	67	165
	2.06	69.5	132
	2.24	85.9	135
	2.32	88.1	145
	3.02	93.5	135
	3.8	99	102
	6	98	114
	10.92	96	140
EPC/Chol/DSPE-PEG_{2000}	1.95	20.6	102
	2	40.9	75
	2.29	74.8	88
	2.35	82.9	95
	2.97	88.8	97
	3.7	97	110
	5.39	98	88
	10.91	97.6	106

P.S. (nm) implied average diameter of liposomes. pH was calculated by the difference in pH before and after titration.

transmembrane pH gradient. For liposomes in 500 mM citrate buffer at pH 3, the external medium was alkalized by adding 1 M NaOH (1 ml/1 ml liposomes). Different pH gradients were produced by adjusting the ratio of citrate buffer to NaOH. Drug uptake was initiated by adding 1 mg of irinotecan to 1 ml of liposomes at a lipid concentration of 5 mM. Then, the solution was vigorously vortexed and incubated in either a 40°C or a 60°C water bath for 10 min. The amount of drug uptake was measured after removal of free drug by size exclusion chromatography (Sephadex G-75; Pharmacia, Uppsala, Sweden).

After drug uptake, liposomes containing irinotecan were transferred to a 37°C waterbath and sampled at different times. Each sample was passed through a Sephadex G-75 column to remove unentrapped drug. After gel filtration, 0.5 ml of a suspension of liposomes was added to a vacuum evaporator to completely remove the water. Then, the concentration of the encapsulated drug was determined by HPLC and calculated from the following equation.

$$\text{Percentage drug uptake (\%)} = \frac{C_t}{C_i} \times 100\%$$

where C_i is the initial concentration of the drug in the liposomes and C_t is the concentration of the drug at the time of sampling.

Drug uptake reached a maximum within 5 min of addition of the drug and was stable for up to 30 min at a low temperature (40°C). Thus, a period of 10 min for loading, and a temperature of 40°C were used in this study. Table 1 summarizes the uptake of irinotecan into liposomes that were composed of EPC/cholesterol or EPC/cholesterol/DSPE-PEG_{2000}. It can be seen that the drug uptake ratio was dependent on the pH gradient. The magnitude of the pH gradient was evaluated by the difference between the pH values before and after alkaline titration. The encapsulation efficiency of both EPC/cholesterol and EPC/cholesterol/DSPE-PEG_{2000} liposomes was 97–99%, and approached a constant when $\Delta pH \geq 3.7$. The presence of 5 mol% DSPE-PEG_{2000} in the liposomes did not enhance the amount of drug uptaken. It was demonstrated that in the absence of a pH gradient, there was no uptake of irinotecan.

A pH gradient of 3.7 was subsequently used to improve the uptake of irinotecan. Figure 1 plots the leakage of irinotecan from liposomes that were composed of EPC/cholesterol, EPC/cholesterol/DSPE-PEG_{2000}, HSPC/cholesterol, HSPC/cholesterol/DSPE-PEG_{2000}, DSPC/cholesterol, and DSPC/cholesterol/DSPE-PEG_{2000} at 37°C as a function of time. Certain amounts of the encapsulated drug (approaching 40%) were released from all liposomes within 20 min and drug leakage followed first-order kinetics during this period. In particular, liposomes that contained EPC of a lower phase transition temperature (approximately −20°C) were found to be highly unstable since their drug encapsulation ratio in liposomes decreased rapidly, and released approximately 95% of the drug uptaken in 240 min. This may be because the lipid membranes consisting of EPC exhibit higher fluidity than membranes consisting of HSPC. In contrast, liposomes that were composed of lipids with higher transition temperatures (about 50–60°C), DSPC and HSPC, were significantly more stable under the experimental conditions used. The percentage of drug remaining approached a steady state in 240 min despite some initial drug release. However, Fig. 1 also shows that adding 5 mol% DSPE-PEG_{2000} into the lipid bilayer under all experimental conditions did not obviously reduce the leakage of irinotecan from liposomes.

The size distributions of EPC/cholesterol, EPC/cholesterol/DSPE-PEG_{2000}, HSPC/cholesterol, HSPC/cholesterol/DSPE-PEG_{2000}, DSPC/cholesterol, and DSPC/cholesterol/DSPE-PEG_{2000} liposomes were recorded at 37°C during the 240-min period. Figure 2 shows the sizes of liposomes, measured by photon correlation spectroscopy. Drug leakage is not clearly related to the size distribution of liposomes, although liposomes containing DSPE-PEG_{2000} are generally smaller than liposomes without DSPE-PEG_{2000}.

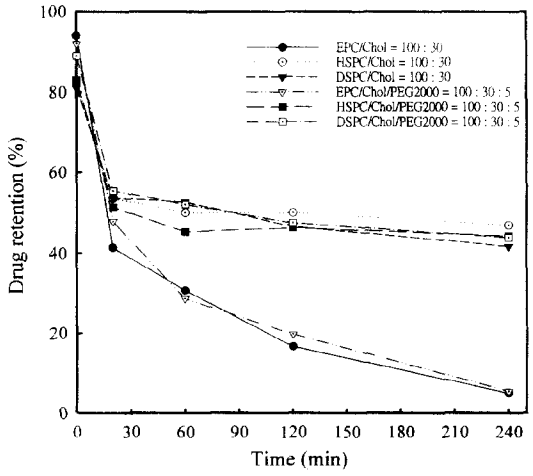

FIG. 1. Drug retention percentage against time for liposomes of different compositions. The standard deviations for these samples were typically <5%. Each data point represents the average of three or more determinations.

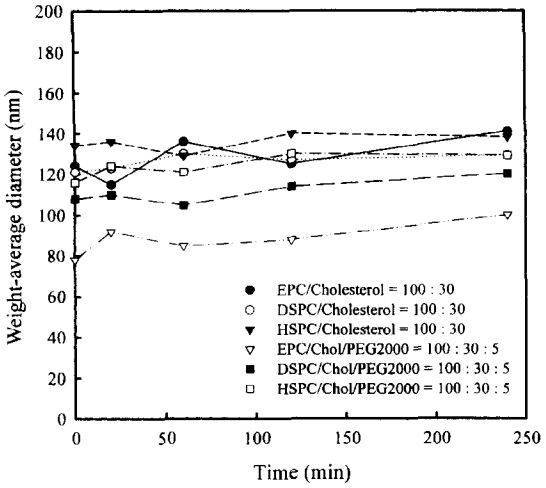

FIG. 2. Particle size (weight-averaged diameter) of liposomes of different compositions versus time. The standard deviations for these samples were typically ±15 nm. Each data point represents the average of three or more determinations.

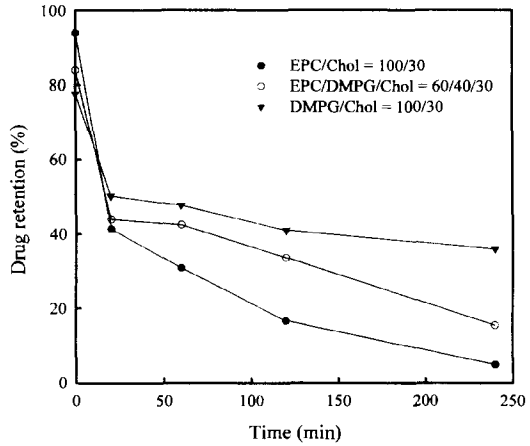

FIG. 3. Drug retention percentage as a function of time for liposomes of EPC/Chol=100/30 (solid circles), EPC/DMPG/Chol=60/40/30 (open circles), and DMPG/Chol=100/30 (triangles). The standard deviations for these samples were typically <5%. Each data point represents the average of three or more determinations.

Previous studies have found that negatively charged compounds could improve the ability of liposomes to encapsulate a basic drug, partly due to the ionic interaction between them. In this study, a negative lipid, DMPG and an anion polymer, dextran sulfate were chosen to be added to lipid vesicles. As shown in Fig. 3, the leakage of the drug was reduced when the proportion of DMPG in liposomes was increased. Similarly, the drug leakage from liposomes was decreased when dextran sulfate was incorporated into the aqueous compartment of liposomes (shown in Fig. 4). Optimal drug maintenance was obtained at 5 mg/ml of dextran sulfate. However, increasing the concentration of dextran sulfate to 20 mg/ml in the hydration buffer caused a foaming instability of the liposome which is why excess dextran sulfate led to decreased retention of the drug. Thus, we recommend that the concentration of dextran sulfate used

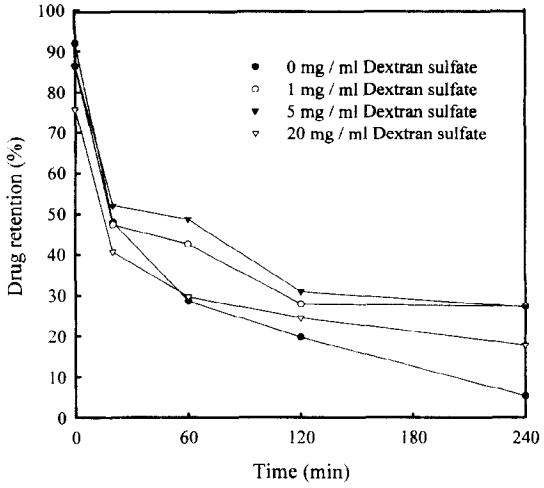

FIG. 4. Drug retention percentage versus time for EPC/Chol/DSPE-PEG$_{2000}$=100/30/5 liposomes for different amounts of added dextran sulfate. The standard deviations for these samples were typically <5%. Each data point represents the average of three or more determinations.

should not exceed 5 mg/ml.

The therapeutic efficacy of many liposomal formulations of membrane-permeable drugs will likely depend on the retention characteristics of the drug. Previous studies of liposomal irinotecan (10, 11) have established that antitumor activity can be significantly improved and the side effects decreased by using liposomes with enhanced drug retention characteristics. The rationale for the experiment described here was that the leakage of irinotecan from liposomes could be significantly reduced by maintaining the incubation temperature at 40°C, because the thermal mobility at a higher temperature (60°C) causes the drug to leak from the liposomes. Of course, it cannot be ruled out that the high temperature causes rapid loss of the pH gradient which reduces the stability of the liposomes.

Results in this study reveal that a transmembrane pH gradient is a prerequisite for transferring irinotecan into liposomes. The uptake of the drug reached a maximum at a ΔpH of 3.7. Irinotecan includes alkaline piperidino groups that display amine functionality. Accordingly, the mechanism of irinotecan transport via a pH gradient is similar to that of many amino group-containing drugs (15). The phenomenon observed in this study has also been recorded by other researchers (14), where by the uptake of free irinotecan into a cell is dependent on pH. Some reports have claimed that irinotecan was incorporated into the hydrophobic section of a lipid bilayer (10, 11) with the amount of the drug encapsulated being around 0.127 mg drug/mg lipid. However, irinotecan is well known to be a water-soluble drug and, according to results in this study, it is trapped into the aqueous compartment of liposomes by the pH gradient method, such that the drug encapsulated could reach amounts as high as 0.254 mg drug/mg lipid. Therefore, even if incorporation into the bilayer cannot be completely ruled out, irinotecan is believed to be distributed primarily in the aqueous compartment of the liposomes.

According to our results, the stability of liposomes was

significantly improved when liposomes were prepared using phospholipids with a high phase transition temperature, such as DSPC and HSPC. Such lipids in liposomes make the membrane more impermeable than the liposomes comprised of EPC. Furthermore, an appropriate proportion of DSPE-PEG is commonly added to liposomes to prevent mononuclear phagocytic system uptake *in vivo* (16). Thus, the influence of adding DSPE-PEG_{2000} on liposomal irinotecan was investigated. Adding 5 mol% DSPE-PEG_{2000} increased the uptake of the drug into liposomes and decreased the average diameter of the liposomes, although it had little effect on the release of the drug. Nevertheless, the size distribution of all liposomes during the study was not significantly changed even during drug release.

The results presented in this paper confirm the prediction that the drug leakage rate could be slightly reduced by incorporating a negatively charged component into liposomes. Although the leakage of drug cannot be completely prevented, DMPG and dextran sulfate both decelerate the leakage of irinotecan (Figs. 3 and 4). This finding can be explained by the fact that irinotecan has the characteristics of a weakly basic drug which electrostatically interacts with the negatively charged membrane. Furthermore, it was also found that sulfated oligosaccharides can form an insoluble complex with irinotecan (data not shown). Consequently, the leakage of the drug was reduced by the generation of such a complex within the liposomeal aqueous compartment. Nevertheless, the stability of liposomes was not obviously improved since the internal lower pH may affect the interaction between dextran sulfate and irinotecan. Another pH gradient method described in the literature using ammonium sulfate, which is a self-sustaining system, should be tried (17).

In summary, liposomes that are composed of HSPC or DSPC with high phase transition temperatures are better carriers for irinotecan than those composed of EPC. Finally, the retention of irinotecan within liposomes has been demonstrated to be slightly increased when the liposomes contain negative lipids or dextran sulfate.

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Levoleucovorin as Replacement for Leucovorin in Cancer Treatment

Victor Tuan Giam Chuang and Manabu Suno

OBJECTIVE: To comprehensively review the literature regarding the efficacy, safety, and costs associated with the use of levoleucovorin in cancer treatment and to assess whether levoleucovorin would be a reasonable alternative to the use of racemic leucovorin.

DATA SOURCES: A MEDLINE search was conducted for English-language human studies published between January 1980 and April 2012 using the terms l-LV, levoleucovorin, d,l-LV, leucovorin, folinic acid, folinate, 5-formyltetrahydrofolate, folic acid, folates, methotrexate, 5-fluorouracil, and clinical trials.

STUDY SELECTION AND DATA EXTRACTION: Articles pertinent to clinical trials (Phase 1, 2, 3) related to evaluating the efficacy, interchangeability, and safety of levoleucovorin were collected and their contents reviewed.

DATA SYNTHESIS: From these pharmacokinetics and clinical studies, information on the use of levoleucovorin as a modulator of fluorouracil as well as when combined with other antitumor agents were scrutinized and extracted for comparison with leucovorin whenever possible. Two randomized Phase 3 clinical studies comparing the efficacy and adverse effect profiles of leucovorin and levoleucovorin demonstrated that levoleucovorin is as effective as leucovorin in terms of response, toxicity, and survival. Six randomized Phase 3 clinical studies demonstrated the safety and efficacy of levoleucovorin as a modulator of fluorouracil in combination with/without other antitumor agents in colorectal cancer patients. Levoleucovorin has been studied in other cancers. These clinical Phase 1/2/3 studies demonstrated efficacy and safety of levoleucovorin in combination chemotherapeutic regimens comprising fluorouracil and other antitumor agents.

CONCLUSIONS: The results of the clinical studies suggest that levoleucovorin is efficacious and can be used safely in combination with fluorouracil and other antitumor agents. Levoleucovorin can be used interchangeably with leucovorin for modulating fluorouracil. The current shortage of the supply of leucovorin centered in North America renders levoleucovorin a reasonable alternative in terms of efficacy and toxicity profile, but from the perspective of cost, leucovorin remains the drug of choice.

KEY WORDS: colorectal cancer, fluorouracil, levoleucovorin, leucovorin, methotrexate.

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Levoleucovorin is the pharmacologically active levoisomer of racemic leucovorin, or folinic acid, a synthetic folate analogue. Levoleucovorin (Fuxilev; Spectrum Pharmaceutical) is available as a ready-to-use solution in 175-mg and 250-mg vials and as a freeze-dried powder in 50-mg vials.[1]

Levoleucovorin was approved by the Food and Drug Administration (FDA) for use as a rescue agent after high-dose methotrexate therapy in treating osteosarcoma to diminish the toxicity and counteract the effects of impaired methotrexate elimination and of the inadvertent overdosage of folic acid antagonists, and in combination with fluorouracil for the palliative treatment of patients with advanced metastatic colorectal cancer.

To date, few reports on the pharmacology, adverse effects, and drug interactions of the biological active levoleucovorin have been published. This could be because levoleucovorin has been shown to be the biologically active isomer; hence, information gathered for leucovorin so far reflects, to a great extent, the properties of levoleucovorin. This notion can be supported by the fact that the d-isomer has been reported to be essentially devoid of pharmacologic activity. Furthermore, although d-levoleucovorin ex-

Author information provided at end of text.

hibits different pharmacokinetic properties, it does not appear to affect the pharmacokinetics of levoleucovorin.

This article reviews the English-language literature regarding the use of levoleucovorin to determine whether levoleucovorin is interchangeable with leucovorin in cancer treatment.

Pharmacology

Folic acid is an essential cofactor for certain enzymes that are involved in the synthesis of purine, thymidine, and methionine.[2] Folates are transported into cells in the form of monoglutamate derivatives and function as a cofactor in 1-carbon transfer reactions. Each 1-carbon form of folate is a required cofactor for 1 or more biosynthetic pathways. To accomplish this, folate must first be reduced by dihydrofolate reductase (DHFR) into the functional metabolic cofactors, dihydrofolate (DHF) and tetrahydrofolate (THF). THF polyglutamates participate in 1-carbon metabolism, where a carbon unit from serine, choline, or glycine is transferred to THF to form methylene-THF, which is either involved in the synthesis of thymidine, which is then incorporated into DNA, oxidized to formyl-THF for the synthesis of purines, or reduced to methyl-THF to form methionine. 5-Formyl-THF (folinic acid, citrovorum factor, leucovorin) is more stable than folate. It does not serve as a metabolic cofactor but may be a storage form of folate.

HIGH-DOSE METHOTREXATE THERAPY

Methotrexate, an antifolate agent, inhibits DHFR and also directly inhibits folate-dependent enzymes that are involved in de novo purine and thymidylate synthesis.[3] In the synthesis of thymidylate, 5,10-methylene-THF is oxidized to DHF. DHF must then be reduced to THF by DHFR to regain its function as a cofactor. Methotrexate prevents the formation of THF through high-affinity binding to DHFR, producing an acute intracellular deficiency of certain folate coenzymes and a vast accumulation of the toxic inhibitory substrate, DHF polyglutamates, as the result of the inhibition of DHFR.[4] This termination of 1-carbon transfer reactions is crucial for the de novo synthesis of purine nucleotides and thymidylate, resulting in the subsequent interruption of the synthesis of DNA and RNA and other vital metabolic reactions.

The toxic effect of methotrexate can be alleviated by leucovorin. Leucovorin antagonises the activity of methotrexate by several mechanisms. It competes with methotrexate for entry into the cell and for folylpolyglutamate synthetase and DHFR enzymes, leading to a reduction in the concentrations of methotrexate polyglutamates and a diminished inhibition of DHFR. Leucovorin does not require reduction by DHFR to participate in reactions in which folates are used as a source of 1-carbon moieties. Moreover, leucovorin is rapidly converted to other reduced folates, thereby restoring the pool of reduced folates. The dextro isomer of leucovorin has been reported to be incapable of reversing the toxicity of methotrexate or potentiating the effect of fluorouracil.[5] On the other hand, levoleucovorin has been shown to be as effective as leucovorin for use as a rescue agent after the administration of high doses of methotrexate in the treatment of osteosarcoma.[5-7] Therefore, levoleucovorin is useful as an antidote to the inhibition of DHFR caused by high doses of methotrexate.

IN COMBINATION WITH FLUOROURACIL

Fluorouracil, a pyrimidine analogue, is metabolized to 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP), which binds to and inhibits thymidylate synthase, an important enzyme in DNA repair and replication. The smaller fluorine group at position 5 of fluorouracil allows the molecule to mimic uracil biochemically, but the fluorine-carbon bond is much stronger than that of C-H and prevents the methylation of the 5 position of fluorouracil by thymidylate synthase. In the presence of the physiologic cofactor 5,10-methylene-THF, fluoropyrimidine locks the enzyme in an inhibited state.[8]

The cytotoxic contribution by levoleucovorin is derived from its ability to potentiate the action of fluorouracil.[9,10] Levoleucovorin is readily converted into 5,10-methylene-THF. This reduced folate acts to stabilize the binding of FdUMP, the active metabolite of fluorouracil, to thymidylate synthase. This stabilization further enhances the inhibition of this enzyme, which is important in DNA repair and replication. For this reason, clinical outcomes in terms of antitumor activity, safety, and the tolerability profiles of fluorouracil are believed to be derived more directly from the cytotoxic agents used rather than the direct action of levoleucovorin on tumors.

Pharmacodynamics and Pharmacokinetics

Intravenous administration of a 25-mg bolus dose or a 15-minute infusion of 300 mg/day of leucovorin showed that levoleucovorin was rapidly cleared from the plasma by conversion to 5-methyl-THF and urinary excretion that can be described by a biexponential function. The urinary clearance of levoleucovorin or 5-methyl-THF differed only slightly from that for creatinine.[11,12] Furthermore, the constant intravenous infusion of large doses of leucovorin 500 mg/m^2 daily for 5.5 days considerably expands the intracellular pools of active folate.[13,14] The mechanisms for the distribution, metabolism, and excretion of leucovorin are not saturable over the dose range of 25-100 mg. However, the conversion of levoleucovorin to 5-methyl-THF was saturable following a 2-hour intravenous infusion of leucovorin 1000 mg, producing equivalent areas under the

curve for levoleucovorin and 5-methyl-THF.[15,16] In contrast, d-levoleucovorin, which is not metabolized and is slowly excreted into the urine, persisted in plasma at concentrations greatly exceeding those of levoleucovorin and 5-methyl-THF.[7,15,17] The low renal clearance of d-levoleucovorin can be attributed to the extensive stereoselective binding of d-levoleucovorin to plasma proteins, particularly human serum albumin.[14,17] This discrepancy in protein binding may influence the biochemical modulation of fluorouracil by THFs because hypoalbuminemia is common in patients with advanced colorectal cancer.[18]

Mader et al.[19] reported 2 significant differences in the kinetics of levoleucovorin when the single isomer was administered via a 2-hour intravenous infusion of levoleucovorin 200 mg/m² with a loading dose of levoleucovorin 100 mg/m². The maximum concentration and area under the curve of the 5-methyl-THF were greater than those observed after an intravenous infusion of leucovorin 400 mg/m² with a leucovorin loading dose of 200 mg/m². Zittoun reported similar observations and concluded that the use of levoleucovorin may prevent the interference of the inactive isomer, especially in patients receiving high doses of leucovorin.[5]

Clinical Studies

INTERCHANGEABILITY OF LEUCOVORIN WITH LEVOLEUCOVORIN AS A MODULATOR OF FLUOROURACIL

The North Central Cancer Treatment Group (Goldberg et al.) carried out a randomized Phase 3 trial involving patients with advanced colorectal cancer in which the efficacy of different forms of leucovorin was examined.[20] This study had 3 arms combining fluorouracil with levoleucovorin, oral leucovorin, or intravenous leucovorin: A (fluorouracil 370 mg/m² and levoleucovorin 100 mg/m²), B (fluorouracil 370 mg/m² and oral leucovorin 125 mg/m²), and C (control, fluorouracil 370 mg/m² and after an intravenous dose of leucovorin 200 mg/m²). Only data related to arms A and arm C were discussed in our article. The most frequent adverse events are listed in Table 1. The adverse events, response, survival, and time to progression were comparable between the 2 arms. Therefore, the available data indicate that levoleucovorin is as effective as leucovorin in terms of response, toxicity, and survival.

Scheithauer et al. published a randomized Phase 3 trial in which the efficacy and adverse effect profile of fluorouracil used with either leucovorin or with levoleucovorin in patients with advanced colorectal cancer were compared.[21] The clinical trial involved treatment with a bolus injection of levoleucovorin or leucovorin at a dose of 100 mg/m² per day, followed by fluorouracil for 5 consecutive days. The dose of leucovorin was the same with both formulations. Thus, patients in the levoleucovorin arm received double the effective dose of leucovorin. The authors found that the 2 arms were not significantly different in terms of efficacy in the leucovorin versus levoleucovorin arms. In addition, no significant difference was found in the 2 arms in terms of patient adherence to chemotherapy and the relative dose intensity of levoleucovorin. However, the incidence of adverse effects was similar in both arms. As such, the 2 randomized studies indicate that levoleucovorin is as effective as leucovorin in terms of response, toxicity, and survival.

SAFETY AND EFFICACY OF LEVOLEUCOVORIN

Levoleucovorin used in trials as a modulator of fluorouracil in combination chemotherapy with/without other antitumor agents are listed in Table 2.[22-28] In these studies, levoleucovorin was used interchangeably with leucovorin. However, no subset analysis was performed to assess whether there was any difference in efficacy and grade 3 or 4 toxicity profiles in patients who received levoleucovorin or leucovorin.

Phase 3 studies on the use of levoleucovorin as a modulator of fluorouracil in combination with irinotecan, raltitrexed, methotrexate, and oxaliplatin[22-28] demonstrated that the maximal benefit of irinotecan plus levoleucovorin-modulated fluorouracil can be obtained in patients with no weight loss, preserved performance status, and a limited

Table 1. Adverse Events Reported with Levoleucovorin and d,l-Leucovorin

Adverse Event	Levoleucovorin/Fluorouracil, n (%) (n = 318)		d,l-Leucovorin/Fluorouracil, n (%) (n = 307)	
	Grade 1-4	Grades 3, 4	Grade 1-4	Grades 3, 4
Stomatitis	229 (72)	37 (12)	221 (72)	44 (14)
Diarrhea	222 (70)	61 (19)	201 (65)	51 (17)
Nausea and vomiting	197 (62)	26 (8)	185 (61)	26 (8)
Abdominal pain	45 (14)	10 (3)	37 (12)	10 (3)
Anorexia/decreased appetite	76 (24)	13 (4)	77 (25)	5 (2)
Dermatitis	91 (29)	3 (1)	88 (29)	4 (1)
Alopecia	83 (26)	1 (0.3)	87 (28)	3 (1)

Table 2. Use of Levoleucovorin in Combination Chemotherapeutic Regimens for Colorectal Cancer

Reference	Cancer Type, (no. of Pts.)	Design	Regimen	Results
Andre (2003)[49]	Colon and high rectum adenocarcinoma, N = 905	Randomized Phase 3	Leucovorin/fluorouracil 2: Levoleucovorin 100 mg/m² (d,l-LV 200 mg/m²) iv 2-hour infusion followed by fluorouracil iv bolus 400 mg/m² and 22-hour iv infusion of 600 mg/m² (repeated every 14 days) FUFOL: Levoleucovorin 100 mg/m² (leucovorin 200 mg/m²) 15-minute infusion followed by fluorouracil 400 mg/m² infusion, repeated daily for 5 days (repeated every 28 days)	OS: not significant (at 41 months of follow-up) Grade 3/4 toxicities: mucositis, neutropenia, and diarrhea seen less with leucovorin/fluorouracil ($p < 0.001$)
Cornella (2000, 2002)[50,51]	Advanced colorectal carcinoma (159)	Randomized Phase 2/3	Arm A day 1: irinotecan 100 mg/m² day 2: levoleucovorin 250 mg/m² and fluorouracil 650 mg/m² bolus Arm B day1: raltitrexed 3 mg/m² day 2: levoleucovorin 250 mg/m² and fluorouracil 1050 mg/m² bolus Arm C day 1: methotrexate 750 mg/m² day 2: levoleucovorin 250 mg/m² and fluorouracil 600 mg/m² bolus	ORR: Arm A: 34%, Arm B: 24%, Arm C: 24% Grade 3/4 toxicities: Arm A: neutropenia: 40%; diarrhea: 18% Arm B: neutropenia: 16%; diarrhea: 16%
Cornella (2005)[28]	Advanced colorectal carcinoma (274)	Randomized Phase 3	(IRIFAFU) day 1: irinotecan 200 mg/m² day 2: levoleucovorin 250 mg/m² and fluorouracil 850 mg/m² bolus (OXAFAFU): day 1: oxaliplatin 100 mg/m² day 2: levoleucovorin 250 mg/m² and fluorouracil 1050 mg/m² bolus	ORR: ($p = 0.029$): IRIFAFU: 31%, OXAFAFU: 44% Grade 3/4 toxicities: IRIFAFU: neutropenia: 31%; diarrhea: 24% OXAFAFU: neutropenia: 29%; diarrhea: 12%
Douillard (2000)[48]	Metastatic colorectal cancer (387)	Randomized Phase 3	Irinotecan: Irinotecan/fluorouracil/calcium folinate No irinotecan: fluorouracil/calcium folinate	RR: Irinotecan: 49%, no irinotecan: 31% OS: Irinotecan:17.4 months, no irinotecan: 14.1 months Grade 3/4 toxicities: significantly more frequent with irinotecan; effects were predictable, reversible, noncumulative, manageable
Tournigand (2004)[27]	Metastatic colorectal cancer (220)	Randomized Phase 3 (FOLFIRI followed by FOLFOX6 or the reverse sequence)	Arm A FOLFIRI (oxaliplatin replaced irinotecan at progression) Arm B FOLFOX6 (irinotecan replaced at progression)	RR: First-line: Arm A: 56%; Arm B: 54% Second-line: Arm A: 15%; Arm B: 4% Grades 3/4 toxicity: First-line: Arm A: neutropenia: 21.8%; diarrhea: 12.7%; mucositis: 9% Arm B: neutropenia: 40%; diarrhea: 10%; mucositis: 1% ($p = 0.26$) Second-line: Similar (mainly neutropenia and diarrhea) Neurological event significantly more frequent in the FOLFOX6
Labianca (2011)[28]	Advanced colorectal carcinoma (337)	Randomized Phase 3	Arm A FOLFIRI (administered every 2 weeks; 2 months on and 2 months off) Arm B FOLFIRI (administered continuously)	OS: Arm A: 18 months, Arm B: 17 months Grades 3/4 toxicity: Similar (mainly myelosuppression, fever and diarrhea)

d,l-LV = d,l-leucovorin; FOLFIRI = fluorouracil/leucovorin/irinotecan; FOLFOX = fluorouracil/ leucovorin/oxaliplatin; FUFOL = fluorouracil/leucovorin or levoleucovorin; IRIFAFU = irinotecan/levoleucovorin/fluorouracil; ORR = overall response rate; OS = overall survival; OXAFAFU = oxaliplatin/levoleucovorin/fluorouracil; RR = response rate.

extent of the disease. This regimen was tolerated well and was found to be efficacious in the elderly.[24]

The efficacy and safety of levoleucovorin in high-dose methotrexate with leucovorin rescue regimens were investigated in a group of 15 patients with osteosarcoma. Adverse effects were mild and myelosuppression was not severe. These results support the conclusion that levoleucovorin effectively rescues patients from the toxicity of high-doses of methotrexate.[29] Similar results and conclusions were reported on the use of levoleucovorin in rescue of high-dose methotrexate therapy in 14 children with acute lymphocytic leukemia.[30] For levoleucovorin or leucovorin rescue, the elimination half-life of methotrexate was similar, 13.9 hours. No significant differences in adverse events such as granulocytes, platelets, transaminase levels, serum creatinine levels, and oral mucositis, were found following levoleucovorin or leucovorin rescue.

From these clinical studies, the use of levoleucovorin as a biochemical modulator of fluorouracil or in high-dose methotrexate therapy rescue demonstrate that levoleucovorin is as efficacious as leucovorin and that no clear increase in the percentage of grade 3 or 4 toxicity profiles when patients were given levoleucovorin.

USE OF LEVOLEUCOVORIN IN OTHER TUMOR CHEMOTHERAPY

In addition to being used in the treatment of colorectal carcinomas, levoleucovorin has been evaluated for use in the treatment of other types of cancer, including head and neck, breast, pancreas, gastric, and neuroendocrine cancers in Phase 1, pharmacokinetic, and Phase 2 clinical trials. These studies are summarized in Table 3.[31-34] The doses of levoleucovorin used in these studies ranged from 100 mg/m^2 to 250 mg/m^2 and were administered in combination with cisplatin and fluorouracil/methotrexate, respectively. The Phase 2 trials showed modest response rates in their tumor types.[31,33,34] The Phase 1 clinical trial reported acceptable tolerability of 250 mg/m^2 of levoleucovorin, with maximum doses of 1050 mg/m^2 with starting doses of 660 mg/m^2 as a bolus dose of fluorouracil and 3 mg/m^2 of raltitrexed.[31] Thus, with promising results from these early trials, to evaluate the role of levoleucovorin in the chemotherapy regimens for these cancers, randomized Phase 3 trials are necessary

Adverse Effects

Reactions to leucovorin appear to be rare. A few cases of leucovorin hypersensitivity reactions have been reported, including a rash during the first treatment course, hypotension and a rash during the second course, urticaria and difficulty in breathing, flushing, hives, body pain, headaches, elevated blood pressures, and general discomfort during the second course[35] (Table 1). Patients receiving levoleucovorin as a rescue agent after high-dose methotrexate therapy have experienced vomiting (38%), stomatitis (38%), and nausea (19%). Diarrhea, nausea, and stomatitis were the most common (>50%) adverse reactions in patients with advanced colorectal cancer receiving levoleucovorin in combination with fluorouracil.[1]

Because of the calcium content of the preparation, no more than 16 mL (160 mg) of levoleucovorin solution should be injected intravenously per minute. Rapid administration of calcium may produce arrhythmia, hypotension, myocardial infarction, and vasodilation. In addition, fluorouracil and levoleucovorin should be administered separately to avoid the formation of a precipitate.[36] Hence, levoleucovorin should not be administered with other therapeutic agents in the same intravenous admixture.

As shown in Table 1, there were no significant differences between levoleucovorin and leucovorin with respect to adverse events.[20]

Table 3. Use of Levoleucovorin in Treating Other Tumor Subtypes

Reference	Tumor type (no. of pts.)	Phase	Treatment	Results
Caponigro (1999)[31]	Advanced head and neck cancer (17)	PK, 1	Day 1: raltitrexed Day 2: levoleucovorin 250 mg/m^2 and escalating bolus of fluorouracil 600 mg/m^2	ORR: 35%, well tolerated
Bajetta (1998)[33]	Advanced breast cancer (70, elderly)	2	Doxifluridine 600 mg/m^2 orally twice daily and levoleucovorin 25 mg every 12 hours for 4 consecutive days every 12 days	RR: 26%, well tolerated
Comella (1998)[32]	Stomach, colorectal, and biliary tract cancers (94)	2	Day 1: methotrexate 500 mg/m^2 2-hour infusion followed by levoleucovorin 250 mg/m^2 2-hour infusion Day 2: fluorouracil 600 mg/m^2 iv bolus, repeated every 2 weeks	RR: 30% in chemotherapy-naive pts.
Artale (2005)[34]	Neuroendocrine (6)	2	Cisplatin 45 mg/m^2, levoleucovorin 100 mg/m^2 over 2 hours and fluorouracil 400 mg/m^2 bolus followed by 600 mg/m^2 over 22 hours, repeated every 14 days	3 PRs

ORR = overall response rate; PK = pharmacokinetic; PR = partial response; RR = response rate.

Drug Interactions

Leucovorin has been reported to decrease the efficacy of phenytoin, possibly by reducing the plasma concentration of phenytoin,[37,38] although leucovorin may be effective in treating neonatal seizures.[39] Other antiepileptic drugs that have been reported to be affected by levoleucovorin include phenobarbital and primidone, where the frequency of seizures increased in susceptible patients.[1] Leucovorin has been reported to potentially interact with trimethoprim-sulfamethoxazole, causing therapeutic failure of the latter in HIV patients with *Pneumocystis jiroveci* pneumonia.[40]

A markedly increased toxicity has been reported when capecitabine, a prodrug of fluorouracil developed for oral administration, was administered after fluorouracil/leucovorin treatment. This is believed to be caused by intracellular folate that is retained after fluorouracil/leucovorin therapy, although the exact mechanism for this is not clear. Leucovorin is known to lower the maximum tolerated dose of fluoropyrimidines, hence making patients more vulnerable to the subsequent fluoropyrimidine regimens with even low-level folate supplementation. Care should be taken when converting therapy from fluorouracil/leucovorin to capecitabine-based regimens.[41] It is not clear to what extent a weekly bolus dose of leucovorin increases folate pools in patients, but it is interesting that such tolerability of fluoropyrimidines shows regional differences. The highest rates of toxicity observed have been reported in the US, possibly resulting from ethnic variation in gene polymorphisms and differences in dietary folic acid intake because of the mandatory fortification of cereal grain products with folic acid to prevent neural tube birth defects, has been in force in the US since 1998.[42] At present, recommendations for a safe washout period or dose reduction for patients switching from fluorouracil/leucovorin to capecitabine are still inconclusive.

Alkalinization of urine and leucovorin administration are implicated in the clinical management of high-dose methotrexate-induced renal dysfunction. Glucarpidase (carboxypeptidase G2) has been reported to be highly effective in rapid and sustained reduction of high concentrations of methotrexate as a result of impaired renal function.[43-45] Glucarpidase is used when unexpected toxicity or renal failure occurs during high-dose methotrexate therapy.[46] The results of an in vitro study suggest that the protective effects of leucovorin can potentially be antagonized by glucarpidase if the drugs are used concurrently.[47] Since leucovorin is a substrate for glucarpidase, the FDA has recommended that leucovorin should not be administered within 2 hours before or after a dose of glucarpidase.[46] A later study reported the beneficial effects of using the combination of glucarpidase and leucovorin in high-dose methotrexate-induced renal dysfunction.[43] However, a similar effect could be achieved with a high dose of leucovorin alone.[48]

Leucovorin has the potential for use as a rescue agent in a few patients treated with raltitrexed, an antifolate thymidylate synthase inhibitor that is not available as a treatment choice in the US, who present a severe pattern of antiproliferative toxicities. Leucovorin competes with raltitrexed for transport and polyglutamation in both tumor and normal tissues, inhibiting further drug uptake and polyglutamation and resulting in the redistribution and/or reduction in the concentration of preformed raltitrexed polyglutamates. The use of leucovorin is not recommended routinely because the antitumor activity of raltitrexed may similarly be reversed.[49]

Dosing and Cost of Levoleucovorin Versus Leucovorin

The d-isomer of leucovorin has no biological activity. Preclinical studies have shown that the d-isomer might compete with the l-isomer for transport into cells.[21,50] Furthermore, the d-isomer is an inhibitor for folylpolyglutamate synthetase, which might cause deleterious effects on the modulation of fluorouracil.[5,21] These reasons provide a scientific rationale for using levoleucovorin, which is devoid of the "unnatural" d-isomer. Ultimately, the sole modification made when using levoleucovorin instead of the racemic form is to administer only half of the dose of the racemic formulation.[7] The dose for levoleucovorin is 50% of the usual dose of leucovorin. For example, 200 mg/m^2 of levoleucovorin, which is equivalent to 400 mg/m^2 of leucovorin, is administered in the FOLFOX6 or FOLFIRI regimen in colorectal cancer. In the widely used FOLFOX6 and FOLFIRI regimens, a patient receives about 400 mg of levoleucovorin, which is equivalent to 800 mg of leucovorin. In Japan, approximately $800 for levoleucovorin and $15 for leucovorin are needed for 1 treatment course of FOLFOX6 and FOLFIRI. This higher cost for treatment courses using levoleucovorin could be because the generic product for levoleucovorin is not available. While there are generic products of leucovorin in the market, none is available for levoleucovorin because the therapeutic composition patent for levoleucovorin will not expire until December 2019.[51]

Discussion

Clinical outcomes with levoleucovorin modulation of fluorouracil are reasonably believed to be derived from the cytotoxic agents themselves, both in terms of antitumor efficacy and adverse event profiles. Goldberg et al. demonstrated that levoleucovorin and leucovorin are equivalent, and one direct clinical pharmacokinetic comparison of levoleucovorin and leucovorin showed no difference in pharmacokinetic parameters.[20]

In our literature review of these studies incorporating levoleucovorin, some European studies used levoleucovorin or leucovorin according to institutional practice, but several others used levoleucovorin exclusively. While

there were studies that included levoleucovorin or leucovorin with fluorouracil only, subsequent studies found favorable efficacy results with additional chemotherapeutic agents, mostly irinotecan and oxaliplatin, but also with the expected hematologic and nonhematologic toxicities. Most of the studies described included patients with colon or rectal cancer, but studies related to gastric, pancreatic, and neuroendocrine tumors have also been published (Table 3).[31-34] Some of the studies involved fluorouracil prodrug, including tegafur-uracil and capecitabine. Other studies also explored the results of double biochemical modulation regimens of fluorouracil, methotrexate, and levoleucovorin. Representing approaches that are advanced from the bolus infusions administered during the 1990s, several studies explored the use of chronomodulation and approaches involving the continuous infusion of modulated fluorouracil-containing chemotherapy regimens.

Summary

This review shows that levoleucovorin has been used interchangeably with leucovorin for modulating fluorouracil in patients with malignancies. There appears to be no significant difference in efficacy or adverse effects between levoleucovorin and leucovorin, regardless of whether they are used in combination with other chemotherapeutic agents. However, care should be taken to prevent prescribing the wrong dose when levoleucovorin is substituted for leucovorin. Furthermore, no more than 16 mL (160 mg) of levoleucovorin solution should be injected intravenously per minute, because of the calcium content of the preparation. In our institution in Japan, the cost of a vial containing 50 mg of levoleucovorin is approximately $200 (USD) compared with a vial containing 50 mg of leucovorin, which costs only $14. The current shortage of leucovorin supply centered in North America renders levoleucovorin a reasonable alternative in terms of efficacy and toxicity profile, but without clear evidence of the correlation of adverse effects to the biological inactive d-isomer of leucovorin, and from the perspective of cost the drug of choice would still be leucovorin.

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Conflict of Interest: Authors reported none

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EXTRACTO

Levoleucovorina Puede Reemplazar Leucovorina en el Tratamiento del Cáncer

VTG Chuang y M Suno

Ann Pharmacother 2012;46:1349-57.

OBJETIVO: El objetivo de este artículo es revisar de forma exhaustiva la literatura existente sobre la eficacia, seguridad, y costes asociados con el uso de levoleucovorina (l-LV) en el tratamiento del cáncer y evaluar si l-LV podría ser una alternativa razonable al uso de leucovorina (LV) racémico.

FUENTES DE INFORMACIÓN: Se llevó a cabo una búsqueda en la base de datos de MEDLINE de estudios escritos en inglés realizados en humanos publicados entre enero de 1980 y abril de 2012 mediante los siguientes términos de búsqueda en inglés: l-LV, levoleucovorina (levoleucovorin), d,l-LV, leucovorina (leucovorin), ácido folínico (folinic acid), folinato (folinate), 5-formiltetrahidrofolato (5-formyltetrahydrofolate), ácido fólico (folic acid), folatos (folates), metotrexato (methotrexate) (MTX), 5-Fluorouracilo (5-Fluorouracil) (5-FU), y ensayos clínicos (clinical trials).

SELECCIÓN DE ESTUDIOS Y EXTRACCIÓN DE DATOS: Se recopilaron artículos pertinentes a ensayos clínicos (fase I, II, III) relacionados con la evaluación de la eficacia, intercambiabilidad y seguridad de l-LV y se revisaron sus contenidos.

SÍNTESIS: De estos estudios farmacocinéticos y clínicos, se examinó la información sobre el uso de l-LV como modulador de 5-FU así como su combinación con otros agentes antitumorales y se extrajo para su comparación con LV siempre que fuera posible. Dos estudios clínicos aleatorizados de fase 3 que comparaban los perfiles de eficacia y efectos adversos de leucovorina y levoleucovorina demostraron que levoleucovorina es tan efectivo como leucovorina en términos de respuesta, toxici-

dad, y supervivencia. Seis estudios clínicos aleatorizados de fase 3 demostraron la seguridad y eficacia de levoleucovorina como modulador del fluroracilo en combinación con o sin otros agentes antitumorales en los pacientes con cáncer colorrectal. Levoleucovorina ha sido objeto de estudio para otros tipos de cáncer. Estos estudios clínicos de fase I, II, y III demostraron la eficacia y seguridad de levoleucovorona en combinación con regímenes de quimioterapia que incluyan fluroracilo y otros agentes antitumorales.

CONCLUSIONES: Los resultados de los estudios clínicos sugieren que I-LV es eficaz y puede emplearse de forma segura en combinación con 5-FU y otros agentes antitumorales. I-LV puede emplearse de forma intercambiable con LV para la modulación de 5-FU. El recorte actual del suministro de LV centrado en Norteamérica convierte a I-LV en una alternativa razonable en términos de eficacia y perfil de toxicidad, pero desde la perspectiva del coste el fármaco de elección seguiría siendo LV.

Traducido por Enrique Muñoz Soler

RÉSUMÉ

Le Lévoleucovorin Pourrait-il Remplacer le Leucovorin en Oncologie

VTG Chuang et M Suno

Ann Pharmacother 2012;46:1349-57.

OBJECTIF: Évaluer les données probantes quant à l'efficacité, l'innocuité, et les coûts associés à l'utilisation du lévoleucovorin dans le traitement de différents cancers et évaluer si ce nouvel agent représente une option raisonnable à l'utilisation du mélange racémique de leucovorin.

SOURCES D'INFORMATION: Une recherche de littérature a été effectuée dans la banque de données MEDLINE (entre les mois de janvier 1980 et avril 2012) en utilisant les mots-clés suivants: lévoleucovorin, isomère lévogyre du leucovorin, mélange racémique de leucovorin, acide folinique, folinate, 5-formyltétrahydrofolate, acide folique, folates, méthotrexate, 5-fluorouracil, et essais cliniques.

SÉLECTION DE L'INFORMATION ET EXTRACTION DES DONNÉES: Tous les essais cliniques de phase I, II, et III ayant évalué l'efficacité, l'innocuité du lévoleucovorin et son interchangeabilité avec le leucovorin ont été revus.

RÉSULTATS: Les informations provenant des essais cliniques et des études pharmacocinétiques ayant documenté l'utilisation du lévoleucovorin comme un agent modulant l'activité du 5-fluorouracil ou en association avec d'autres agents anti-tumeur sont présentées et discutées. Les résultats de 2 essais cliniques de phase III à répartition aléatoire suggèrent que le lévoleucovorin démontre une efficacité, une réponse clinique, une toxicité, et des données de survie similaires à celles du leucovorin. L'efficacité et l'innocuité du lévoleucovorin comme un agent de modulation possible dans le traitement par le 5-fluorouracil, en association ou non avec d'autres agents antitumoraux chez les patients souffrant de cancer colorectal, ont aussi été documentés dans 6 essais cliniques de phase III. Finalement, l'utilisation du lévoleucovorin dans d'autres types de cancer, en association avec des traitements à base de fluorouracil et d'autres agents antitumoraux, est décrites dans différentes publications de phase I, II, et III.

CONCLUSIONS: Le lévoleucovorin est efficace et peut être utilisé de façon sécuritaire en combinaison avec le 5-fluorouracil et d'autres agents anti-tumeur. Le lévoleucovorin semble pouvoir être interchangeable avec le leucovorin pour moduler l'activité du 5-fluorouracil. Considérant son profil d'efficacité et d'innocuité, le lévoleucovorin pourrait s'avérer une alternative raisonnable durant la pénurie nord-américaine de leucovorin. Son coût est toutefois plus élevé que le leucovorin.

Traduit par Sylvie Robert

View of NCT00813163 on 2011_01_11

ClinicalTrials Identifier: NCT00813163
Updated: 2011_01_11

Descriptive Information

Brief title Study of PEP02 as a Second Line Therapy for Metastatic Pancreatic Cancer

Official title A Phase II Study of PEP02 as a Second Line Therapy for Patients With Metastatic Pancreatic Cancer

Brief summary

The purpose of this study is to see the effect of PEP02 in the treatment of metastatic pancreatic cancer.

Detailed description

Gemcitabine monotherapy or a gemcitabine-based combination regimen is the standard first line therapy for advanced pancreatic cancer. After disease progression, there is no standard treatment available. In animal studies and a previous phase I trial, PEP02 has shown anti-tumor activity and preliminary efficacy in pancreatic cancer. In addition, a phase II study of free-form irinotecan single agent has already shown encouraging activity as second-line treatment for patients with advanced pancreatic cancer refractory to gemcitabine. The liposome formulation of PEP02 theoretically has therapeutic advantages over free-form irinotecan, such as site-specific delivery and extended release of drug. Hence PEP02 may be able to provide better efficacy than free-form irinotecan.

The primary purpose of this phase II study is to evaluate the activity of PEP02 as a second-line therapy in patients with metastatic pancreatic cancer failed to gemcitabine treatment. The primary goal is to measure the 3-month survival rate. An optimal Simon's 2-stage design will be used for this exploratory phase II study.

Phase Phase 2
Study type Interventional
Study design Treatment
Study design Non-Randomized
Study design Open Label
Study design Single Group Assignment
Study design Efficacy Study
Primary outcome

	Measure: Survival Rate Time Frame: 3-month Safety Issue? No
Secondary outcome	Measure: other efficacy endpoints such as objective tumor response, PFS, duration of response, overall survival, tumor marker response of CA19-9, clinical benefit response Safety Issue? No
Secondary outcome	Measure: toxicities Safety Issue? Yes
Secondary outcome	Measure: pharmacogenetics Safety Issue? No
Enrollment	40 (Actual)
Condition	Pancreatic Neoplasms
Arm/Group	Arm Label: PEP02 Experimental Liposome Irinotecan
Intervention	Drug: PEP02 Arm Label: PEP02 120 mg/m2, IV infusion for 90 minutes on day 1 of each 21 days as a treatment cycle. Number of Cycles: until progression or unacceptable toxicity develops.

Recruitment Information

Status	Active, not recruiting
Start date	2009-01
Last follow-up date	2011-06 (Anticipated)
Primary completion date	2010-09 (Actual)

Criteria

Inclusion Criteria:
- Histologically or cytologically confirmed adenocarcinoma of exocrine pancreas
- Metastatic disease
- Documented disease progression after treatment with 1 line of prior gemcitabine-based regimen
- Karnofsky performance status equal or more than 70

Exclusion Criteria:
- With active CNS metastases
- With clinically significant gastrointestinal disorder (e.g., bleeding, inflammation, occlusion, or diarrhea > grade 1)
- Major surgery or radiotherapy within 4 weeks
- Prior participation in any investigational drug study within 4 weeks
- With prior irinotecan treatment

Gender	Both
Minimum age	18 Years

Healthy volunteers	No

Administrative Data

Organization name	PharmaEngine
Organization study ID	PEP0208
Sponsor	PharmaEngine
Health Authority	United States: Food and Drug Administration
Health Authority	Taiwan: Department of Health

View of NCT00813163 on 2012_03_01

ClinicalTrials Identifier: NCT00813163
Updated: 2012_03_01

Descriptive Information

Brief title Study of PEP02 as a Second Line Therapy for Metastatic Pancreatic Cancer

Official title A Phase II Study of PEP02 as a Second Line Therapy for Patients With Metastatic Pancreatic Cancer

Brief summary

The purpose of this study is to see the effect of PEP02 in the treatment of metastatic pancreatic cancer.

Detailed description

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Phase Phase 2
Study type Interventional
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Primary outcome

	Measure: Survival Rate Time Frame: 3-month Safety Issue? No
Secondary outcome	Measure: other efficacy endpoints such as objective tumor response, PFS, duration of response, overall survival, tumor marker response of CA19-9, clinical benefit response Safety Issue? No
Secondary outcome	Measure: toxicities Safety Issue? Yes
Secondary outcome	Measure: pharmacogenetics Safety Issue? No
Enrollment	40 (Actual)
Condition	Pancreatic Neoplasms
Arm/Group	Arm Label: PEP02 Experimental Liposome Irinotecan
Intervention	Drug: PEP02 Arm Label: PEP02 120 mg/m2, IV infusion for 90 minutes on day 1 of each 21 days as a treatment cycle. Number of Cycles: until progression or unacceptable toxicity develops.

Recruitment Information

Status	Active, not recruiting
Start date	2009-01
Last follow-up date	2012-05 (Anticipated)
Primary completion date	2010-12 (Actual)

Criteria

Inclusion Criteria:
- Histologically or cytologically confirmed adenocarcinoma of exocrine pancreas
- Metastatic disease
- Documented disease progression after treatment with 1 line of prior gemcitabine-based regimen
- Karnofsky performance status equal or more than 70

Exclusion Criteria:
- With active CNS metastases
- With clinically significant gastrointestinal disorder (e.g., bleeding, inflammation, occlusion, or diarrhea > grade 1)
- Major surgery or radiotherapy within 4 weeks
- Prior participation in any investigational drug study within 4 weeks
- With prior irinotecan treatment

Gender	Both
Minimum age	18 Years

Healthy volunteers	No

Administrative Data

Organization name	PharmaEngine
Organization study ID	PEP0208
Sponsor	PharmaEngine
Health Authority	United States: Food and Drug Administration
Health Authority	Taiwan: Department of Health

Study of PEP02 as a Second Line Therapy for Metastatic Pancreatic Cancer

This study has been completed.

Sponsor:
PharmaEngine

ClinicalTrials.gov Identifier:
NCT00813163

First Posted: December 22, 2008
Last Update Posted: April 6, 2017

Information provided by (Responsible Party):
PharmaEngine

Full Text View | Tabular View | No Study Results Posted | Disclaimer
How to Read a Study Record

Purpose

The purpose of this study is to see the effect of PEP02 in the treatment of metastatic pancreatic cancer.

Condition	Intervention	Phase
Pancreatic Neoplasms	Drug: PEP02	Phase 2

Study Type:	Interventional
Study Design:	Intervention Model: Single Group Assignment Masking: None (Open Label) Primary Purpose: Treatment
Official Title:	A Phase II Study of PEP02 as a Second Line Therapy for Patients With Metastatic Pancreatic Cancer

Resource links provided by NLM:

MedlinePlus related topics: Pancreatic Cancer

Drug Information available for: Irinotecan Irinotecan hydrochloride

U.S. FDA Resources

Further study details as provided by PharmaEngine:

Primary Outcome Measures:

- Survival Rate [Time Frame: 3-month]

Secondary Outcome Measures:

- other efficacy endpoints such as objective tumor response, PFS, duration of response, overall survival, tumor marker response of CA19-9, clinical benefit response
- toxicities
- pharmacogenetics

Enrollment:	41
Study Start Date:	January 2009
Study Completion Date:	July 2012
Primary Completion Date:	December 2010 (Final data collection date for primary outcome measure)

Arms	Assigned Interventions
Experimental: PEP02	Drug: PEP02

Liposome Irinotecan	120 mg/m2, IV infusion for 90 minutes on day 1 of each 21 days as a treatment cycle. Number of Cycles: until progression or unacceptable toxicity develops. Other Name: Liposome irinotecan

Detailed Description:

Gemcitabine monotherapy or a gemcitabine-based combination regimen is the standard first line therapy for advanced pancreatic cancer. After disease progression, there is no standard treatment available. In animal studies and a previous phase I trial, PEP02 has shown anti-tumor activity and preliminary efficacy in pancreatic cancer. In addition, a phase II study of free-form irinotecan single agent has already shown encouraging activity as second-line treatment for patients with advanced pancreatic cancer refractory to gemcitabine. The liposome formulation of PEP02 theoretically has therapeutic advantages over free-form irinotecan, such as site-specific delivery and extended release of drug. Hence PEP02 may be able to provide better efficacy than free-form irinotecan.

The primary purpose of this phase II study is to evaluate the activity of PEP02 as a second-line therapy in patients with metastatic pancreatic cancer failed to gemcitabine treatment. The primary goal is to measure the 3-month survival rate. An optimal Simon's 2-stage design will be used for this exploratory phase II study.

Eligibility

Information from the National Library of Medicine NIH NLM

Choosing to participate in a study is an important personal decision. Talk with your doctor and family members or friends about deciding to join a study. To learn more about this study, you or your doctor may contact the study research staff using the contacts provided below. For general information, Learn About Clinical Studies.

Ages Eligible for Study:	18 Years and older (Adult, Senior)
Sexes Eligible for Study:	All
Accepts Healthy Volunteers:	No

Criteria

Inclusion Criteria:

- Histologically or cytologically confirmed adenocarcinoma of exocrine pancreas
- Metastatic disease
- Documented disease progression after treatment with 1 line of prior gemcitabine-based regimen
- Karnofsky performance status equal or more than 70

Exclusion Criteria:

- With active CNS metastases
- With clinically significant gastrointestinal disorder (e.g., bleeding, inflammation, occlusion, or diarrhea > grade 1)
- Major surgery or radiotherapy within 4 weeks
- Prior participation in any investigational drug study within 4 weeks
- With prior irinotecan treatment

Contacts and Locations

Information from the National Library of Medicine NIH NLM

To learn more about this study, you or your doctor may contact the study research staff using the contact information provided by the sponsor.

Please refer to this study by its ClinicalTrials.gov identifier (NCT number): ***NCT00813163***

Locations

United States, California

Comprehensive Cancer Center, UCSF
San Francisco, California, United States, 94115

Taiwan

National Health Research Institutes/National Chen-Kung Uiversity Hospital
Tainan, Taiwan, 704

National Taiwan University Hospital
Taipei, Taiwan, 100

Sponsors and Collaborators

PharmaEngine

Investigators

Principal Investigator:	Li-Tzong Chen, M.D.	National Health Research Institutes, Taiwan
Principal Investigator:	Andrew H Ko, M.D.	University of California, San Francisco
Principal Investigator:	Yu-Lin Lin, M.D.	National Taiwan University Hospital

More Information

Publications automatically indexed to this study by ClinicalTrials.gov Identifier (NCT Number):

Ko AH, Tempero MA, Shan YS, Su WC, Lin YL, Dito E, Ong A, Wang YW, Yeh CG, Chen LT. A multinational phase 2 study of nanoliposomal irinotecan sucrosofate (PEP02, MM-398) for patients with gemcitabine-refractory metastatic pancreatic cancer. Br J Cancer. 2013 Aug 20;109(4):920-5. doi: 10.1038/bjc.2013.408. Epub 2013 Jul 23.

Responsible Party:	PharmaEngine
ClinicalTrials.gov Identifier:	NCT00813163 History of Changes
Other Study ID Numbers:	PEP0208
First Submitted:	December 18, 2008
First Posted:	December 22, 2008
Last Update Posted:	April 6, 2017
Last Verified:	January 2015

Keywords provided by PharmaEngine:
Phase II study
Second line
Pancreatic cancer
Metastatic

Additional relevant MeSH terms:

Pancreatic Neoplasms	Irinotecan
Digestive System Neoplasms	Antineoplastic Agents, Phytogenic

Neoplasms by Site
Neoplasms
Endocrine Gland Neoplasms
Digestive System Diseases
Pancreatic Diseases
Endocrine System Diseases

Antineoplastic Agents
Topoisomerase I Inhibitors
Topoisomerase Inhibitors
Enzyme Inhibitors
Molecular Mechanisms of Pharmacological Action

View of NCT00940758 on 2009_07_16

ClinicalTrials Identifier: NCT00940758
Updated: 2009_07_16

Descriptive Information

Brief title Pharmacokinetic Study of Biweekly PEP02 (Liposome Irinotecan) in Patients With Metastatic Colorectal Cancer Refractory to First-line Oxaliplatin-based Chemotherapy

Official title

Brief summary

The purpose of this study is to evaluate the dose-limiting toxicity (DLT), toxicity profile, maximum tolerated dose (MTD) and characterize the pharmacokinetics of biweekly PEP02 treatment.

Detailed description

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: To evaluate the DLT and the toxicity profile Time Frame: 3 years Safety Issue? No
Primary outcome	Measure: To establish the MTD Time Frame: 3 years Safety Issue? No
Primary outcome	Measure: To characterize the pharmacokinetics of biweekly PEP02 in patients with metastatic colorectal cancer who failed to first-line oxaliplatin-based chemotherapy Time Frame: 3 years Safety Issue? No
Secondary outcome	Measure: To collect data for preliminary evaluation of tumor response Time Frame: 3 years Safety Issue? No
Secondary outcome	Measure: To explore the association of the pharmacogenetics of PEP02 including UGT1A family -

	UGT1A1 and UGT1A9 with pharmacokinetic parameters and toxicity Time Frame: 3 years Safety Issue? No
Enrollment	30 (Anticipated)
Condition	Metastatic Colorectal Cancer
Arm/Group	Arm Label: PEP02 Experimental
Intervention	Drug: PEP02 Arm Label: PEP02 Dose escalation: 50-100 mg/m2 biweekly

Recruitment Information

Status	Recruiting
Start date	2009-06
Last follow-up date	2009-12 (Anticipated)
Primary completion date	2009-12 (Anticipated)

Criteria

Inclusion Criteria:
- Histopathologically confirmed metastatic colorectal cancer
- Documented disease progression after first-line chemotherapy containing oxaliplatin
- Both genders, age 18 years
- ECOG performance status 0 or 1
- Adequate organ and marrow function
- Written informed consent to participate in the study

Exclusion Criteria:
- Have received irinotecan treatment
- With active CNS metastases (indicated by clinical symptoms, cerebral edema, steroid requirement, or progressive growth)
- With clinically significant gastrointestinal disorder (e.g. bleeding, inflammation, obstruction, including partial or complete obstruction secondary to peritoneal carcinomatosis, or diarrhea > grade 1)
- With uncontrolled intercurrent illness that could limit study compliance considered to be ineligible for the study by the investigators including, but NOT limited to, any of the following:ongoing or active infection requiring antibiotic treatment, symptomatic congestive heart failure, unstable angina pectoris, or cardiac arrhythmia psychiatric illness or social situation that would preclude study compliance
- With other primary malignancies, except those remain disease-free for 3 or more years after curative treatment.
- Prior chemotherapy within 3 weeks
- Major surgery or radiotherapy within 4 weeks
- Prior participation in any investigational drug study within 3 weeks
- History of allergic reaction to liposome product
- Pregnant or breastfeeding (a urine pregnancy test must be performed on all

patients who are of childbearing potential before entering the study, and the result must be negative)

Gender	Both
Minimum age	18 Years
Healthy volunteers	No

Administrative Data

Organization name	PharmaEngine
Organization study ID	PIST-CRC-01
Sponsor	PharmaEngine
Health Authority	Taiwan: Department of Health

View of NCT00940758 on 2010_02_03

ClinicalTrials Identifier: NCT00940758
Updated: 2010_02_03

Descriptive Information

Brief title Phase I and Pharmacokinetic Study of Biweekly PEP02 in mCRC Refractory to 1st-line Oxaliplatin Base Therapy

Official title Phase I and Pharmacokinetic Study of Biweekly PEP02 (Liposome Irinotecan) in Patients With Metastatic Colorectal Cancer Refractory to First-line Oxaliplatin-based Chemotherapy

Brief summary

The purpose of this study is to evaluate the dose-limiting toxicity (DLT), toxicity profile, maximum tolerated dose (MTD) and characterize the pharmacokinetics of biweekly PEP02 treatment.

Detailed description

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: To evaluate the DLT and the toxicity profile Time Frame: 3 years Safety Issue? No
Primary outcome	Measure: To establish the MTD Time Frame: 3 years Safety Issue? No
Primary outcome	Measure: To characterize the pharmacokinetics of biweekly PEP02 in patients with metastatic colorectal cancer who failed to first-line oxaliplatin-based chemotherapy Time Frame: 3 years Safety Issue? No
Secondary outcome	Measure: To collect data for preliminary evaluation of tumor response Time Frame: 3 years Safety Issue? No

Secondary outcome	Measure: To explore the association of the pharmacogenetics of PEP02 including UGT1A family - UGT1A1 and UGT1A9 with pharmacokinetic parameters and toxicity Time Frame: 3 years Safety Issue? No
Enrollment	30 (Anticipated)
Condition	Metastatic Colorectal Cancer
Arm/Group	Arm Label: PEP02 Experimental
Intervention	Drug: PEP02 Arm Label: PEP02 Dose escalation: 50-100 mg/m2 biweekly

Recruitment Information

Status	Recruiting
Start date	2009-06
Last follow-up date	2010-06 (Anticipated)
Primary completion date	2010-06 (Anticipated)

Criteria

Inclusion Criteria:
- Histopathologically confirmed metastatic colorectal cancer
- Documented disease progression after first-line chemotherapy containing oxaliplatin
- Both genders, age 18 years
- ECOG performance status 0 or 1
- Adequate organ and marrow function
- Written informed consent to participate in the study

Exclusion Criteria:
- Have received irinotecan treatment
- With active CNS metastases (indicated by clinical symptoms, cerebral edema, steroid requirement, or progressive growth)
- With clinically significant gastrointestinal disorder (e.g. bleeding, inflammation, obstruction, including partial or complete obstruction secondary to peritoneal carcinomatosis, or diarrhea > grade 1)
- With uncontrolled intercurrent illness that could limit study compliance considered to be ineligible for the study by the investigators including, but NOT limited to, any of the following:ongoing or active infection requiring antibiotic treatment, symptomatic congestive heart failure, unstable angina pectoris, or cardiac arrhythmia psychiatric illness or social situation that would preclude study compliance
- With other primary malignancies, except those remain disease-free for 3 or more years after curative treatment.
- Prior chemotherapy within 3 weeks
- Major surgery or radiotherapy within 4 weeks
- Prior participation in any investigational drug study within 3 weeks

- History of allergic reaction to liposome product
- Pregnant or breastfeeding (a urine pregnancy test must be performed on all patients who are of childbearing potential before entering the study, and the result must be negative)

Gender	Both
Minimum age	18 Years
Healthy volunteers	No

Administrative Data

Organization name	PharmaEngine
Organization study ID	PIST-CRC-01
Sponsor	PharmaEngine
Health Authority	Taiwan: Department of Health

Trial record 1 of 9 for: pep02
Previous Study | Return to List | Next Study

Phase I and Pharmacokinetic Study of Biweekly PEP02 in mCRC Refractory to 1st-line Oxaliplatin Base Therapy

The recruitment status of this study is unknown. The completion date has passed and the status has not been verified in more than two years.

Verified March 2012 by PharmaEngine.
Recruitment status was: Active, not recruiting

Sponsor:
PharmaEngine

Information provided by:
PharmaEngine

ClinicalTrials.gov Identifier:
NCT00940758

First received: July 15, 2009
Last updated: March 1, 2012
Last verified: March 2012
History of Changes

Full Text View | Tabular View | No Study Results Posted | Disclaimer | How to Read a Study Record

Purpose

The purpose of this study is to evaluate the dose-limiting toxicity (DLT), toxicity profile, maximum tolerated dose (MTD) and characterize the pharmacokinetics of biweekly **PEP02** treatment.

Condition	Intervention	Phase
Metastatic Colorectal Cancer	Drug: **PEP02**	Phase 1

Study Type: Interventional
Study Design: Endpoint Classification: Safety/Efficacy Study
Intervention Model: Single Group Assignment
Masking: Open Label
Primary Purpose: Treatment

Official Title: Phase I and Pharmacokinetic Study of Biweekly **PEP02** (Liposome Irinotecan) in Patients With Metastatic Colorectal Cancer Refractory to First-line Oxaliplatin-based Chemotherapy

Resource links provided by NLM:

MedlinePlus related topics: Colorectal Cancer

Drug Information available for: Oxaliplatin

U.S. FDA Resources

Further study details as provided by PharmaEngine:

Primary Outcome Measures:

- To evaluate the DLT and the toxicity profile [Time Frame: 3 years] [Designated as safety issue: No]
- To establish the MTD [Time Frame: 3 years] [Designated as safety issue: No]
- To characterize the pharmacokinetics of biweekly **PEP02** in patients with metastatic colorectal cancer who failed to first-line oxaliplatin-based chemotherapy [Time Frame: 3 years] [Designated as safety issue: No]

Secondary Outcome Measures:

- To collect data for preliminary evaluation of tumor response [Time Frame: 3 years] [Designated as safety issue: No]

- To explore the association of the pharmacogenetics of **PEP02** including UGT1A family - UGT1A1 and UGT1A9 with pharmacokinetic parameters and toxicity [Time Frame: 3 years] [Designated as safety issue: No]

Enrollment:	18
Study Start Date:	June 2009
Estimated Study Completion Date:	June 2012
Primary Completion Date:	September 2011 (Final data collection date for primary outcome measure)

Arms	Assigned Interventions
Experimental: **PEP02**	Drug: **PEP02** Dose escalation: 50-100 mg/m2 biweekly

Eligibility

Ages Eligible for Study:	18 Years and older (Adult, Senior)
Genders Eligible for Study:	Both
Accepts Healthy Volunteers:	No

Criteria

Inclusion Criteria:

- Histopathologically confirmed metastatic colorectal cancer
- Documented disease progression after first-line chemotherapy containing oxaliplatin
- Both genders, age 18 years
- ECOG performance status 0 or 1
- Adequate organ and marrow function
- Written informed consent to participate in the study

Exclusion Criteria:

- Have received irinotecan treatment
- With active CNS metastases (indicated by clinical symptoms, cerebral edema, steroid requirement, or progressive growth)
- With clinically significant gastrointestinal disorder (e.g. bleeding, inflammation, obstruction, including partial or complete obstruction secondary to peritoneal carcinomatosis, or diarrhea > grade 1)
- With uncontrolled intercurrent illness that could limit study compliance considered to be ineligible for the study by the investigators including, but NOT limited to, any of the following:ongoing or active infection requiring antibiotic treatment, symptomatic congestive heart failure, unstable angina pectoris, or cardiac arrhythmia psychiatric illness or social situation that would preclude study compliance
- With other primary malignancies, except those remain disease-free for 3 or more years after curative treatment.
- Prior chemotherapy within 3 weeks
- Major surgery or radiotherapy within 4 weeks
- Prior participation in any investigational drug study within 3 weeks
- History of allergic reaction to liposome product
- Pregnant or breastfeeding (a urine pregnancy test must be performed on all patients who are of childbearing potential before entering the study, and the result must be negative)

Contacts and Locations

Choosing to participate in a study is an important personal decision. Talk with your doctor and family members or friends about deciding to join a study. To learn more about this study, you or your doctor may contact the study research staff using the Contacts provided below. For general information, see Learn About Clinical Studies.

Please refer to this study by its ClinicalTrials.gov identifier: NCT00940758

Locations

Taiwan

National Cheng Kung University Hospital
Tainan, Taiwan, 704

Sponsors and Collaborators

PharmaEngine

More Information

Responsible Party:	Jang-Yang Chang, National Health Research Institutes
ClinicalTrials.gov Identifier:	NCT00940758 History of Changes
Other Study ID Numbers:	PIST-CRC-01
Study First Received:	July 15, 2009
Last Updated:	March 1, 2012
Health Authority:	Taiwan: Department of Health

Additional relevant MeSH terms:

- Colorectal Neoplasms
- Intestinal Neoplasms
- Gastrointestinal Neoplasms
- Digestive System Neoplasms
- Neoplasms by Site
- Neoplasms
- Digestive System Diseases
- Gastrointestinal Diseases
- Colonic Diseases
- Intestinal Diseases
- Rectal Diseases
- Oxaliplatin
- Antineoplastic Agents

ClinicalTrials.gov processed this record on November 30, 2016

Phase I and Pharmacokinetic Study of Biweekly PEP02 in mCRC Refractory to 1st-line Oxaliplatin Base Therapy

This study has been completed.

Sponsor:
PharmaEngine

ClinicalTrials.gov Identifier:
NCT00940758

First Posted: July 16, 2009
Last Update Posted: April 6, 2017

Information provided by (Responsible Party):
PharmaEngine

Full Text View | Tabular View | No Study Results Posted | Disclaimer
How to Read a Study Record

Purpose

The purpose of this study is to evaluate the dose-limiting toxicity (DLT), toxicity profile, maximum tolerated dose (MTD) and characterize the pharmacokinetics of biweekly PEP02 treatment.

Condition	Intervention	Phase

Metastatic Colorectal Cancer	Drug: PEP02	Phase 1

Study Type: Interventional

Study Design: Intervention Model: Single Group Assignment
Masking: None (Open Label)
Primary Purpose: Treatment

Official Title: Phase I and Pharmacokinetic Study of Biweekly PEP02 (Liposome Irinotecan) in Patients With Metastatic Colorectal Cancer Refractory to First-line Oxaliplatin-based Chemotherapy

Resource links provided by NLM:

MedlinePlus related topics: Colorectal Cancer

Drug Information available for: Oxaliplatin

U.S. FDA Resources

Further study details as provided by PharmaEngine:

Primary Outcome Measures:

- To evaluate the DLT and the toxicity profile [Time Frame: 3 years]
- To establish the MTD [Time Frame: 3 years]
- To characterize the pharmacokinetics of biweekly PEP02 in patients with metastatic colorectal cancer who failed to first-line oxaliplatin-based chemotherapy [Time Frame: 3 years]

Secondary Outcome Measures:

- To collect data for preliminary evaluation of tumor response [Time Frame: 3 years]
- To explore the association of the pharmacogenetics of PEP02 including UGT1A family - UGT1A1 and UGT1A9 with pharmacokinetic parameters and toxicity [Time Frame: 3 years]

Enrollment: 18
Actual Study Start Date: June 2009
Study Completion Date: June 2014
Primary Completion Date: May 2012 (Final data collection date for primary outcome measure)

Arms	Assigned Interventions
Experimental: PEP02	Drug: PEP02 Dose escalation: 50-100 mg/m2 biweekly

Eligibility

Information from the National Library of Medicine NIH NLM

Choosing to participate in a study is an important personal decision. Talk with your doctor and family members or friends about deciding to join a study. To learn more about this study, you or your doctor may contact the study research staff using the contacts provided below. For general information, Learn About Clinical Studies

Ages Eligible for Study: 18 Years and older (Adult, Senior)
Sexes Eligible for Study: All
Accepts Healthy Volunteers: No

Criteria

Inclusion Criteria:

- Histopathologically confirmed metastatic colorectal cancer
- Documented disease progression after first-line chemotherapy containing oxaliplatin
- Both genders, age 18 years
- ECOG performance status 0 or 1
- Adequate organ and marrow function
- Written informed consent to participate in the study

Exclusion Criteria:

- Have received irinotecan treatment
- With active CNS metastases (indicated by clinical symptoms, cerebral edema, steroid requirement, or progressive growth)

- With clinically significant gastrointestinal disorder (e.g. bleeding, inflammation, obstruction, including partial or complete obstruction secondary to peritoneal carcinomatosis, or diarrhea > grade 1)
- With uncontrolled intercurrent illness that could limit study compliance considered to be ineligible for the study by the investigators including, but NOT limited to, any of the following:ongoing or active infection requiring antibiotic treatment, symptomatic congestive heart failure, unstable angina pectoris, or cardiac arrhythmia psychiatric illness or social situation that would preclude study compliance
- With other primary malignancies, except those remain disease-free for 3 or more years after curative treatment.
- Prior chemotherapy within 3 weeks
- Major surgery or radiotherapy within 4 weeks
- Prior participation in any investigational drug study within 3 weeks
- History of allergic reaction to liposome product
- Pregnant or breastfeeding (a urine pregnancy test must be performed on all patients who are of childbearing potential before entering the study, and the result must be negative)

Contacts and Locations

Information from the National Library of Medicine NIH NLM

To learn more about this study, you or your doctor may contact the study research staff using the contact information provided by the sponsor.

Please refer to this study by its ClinicalTrials.gov identifier (NCT number): ***NCT00940758***

Locations

Taiwan

National Cheng Kung University Hospital
Tainan, Taiwan, 704

Sponsors and Collaborators

PharmaEngine

More Information

Responsible Party:	PharmaEngine
ClinicalTrials.gov Identifier:	NCT00940758 History of Changes
Other Study ID Numbers:	PIST-CRC-01
First Submitted:	July 15, 2009
First Posted:	July 16, 2009
Last Update Posted:	April 6, 2017
Last Verified:	April 2017

Additional relevant MeSH terms:

- Colorectal Neoplasms
- Intestinal Neoplasms
- Gastrointestinal Neoplasms
- Digestive System Neoplasms
- Neoplasms by Site
- Neoplasms
- Digestive System Diseases
- Gastrointestinal Diseases
- Colonic Diseases
- Intestinal Diseases
- Rectal Diseases
- Oxaliplatin
- Antineoplastic Agents

View of NCT01375816 on 2011_06_16

ClinicalTrials Identifier: NCT01375816
Updated: 2011_06_16

Descriptive Information

Brief title Liposome-encapsulated Irinotecan Hydrochloride PEP02 or Irinotecan Hydrochloride, Leucovorin Calcium, and Fluorouracil as Second-Line Therapy in Treating Patients With Metastatic Colorectal Cancer

Official title A Randomized Phase II Study of PEP02 or Irinotecan in Combination With Leucovorin and 5-Flourouracil in Second Line Therapy of Metastatic Colorectal Cancer

Brief summary

RATIONALE: Drugs used in chemotherapy, such as liposome-encapsulated irinotecan hydrochloride PEP02, irinotecan hydrochloride, leucovorin calcium, and fluorouracil, work in different ways to stop the growth of tumor cells, either by killing the cells or by stopping them from dividing. It is not yet known whether giving liposome-encapsulated irinotecan hydrochloride PEP02 together with leucovorin calcium and fluorouracil is more effective than giving irinotecan hydrochloride together with leucovorin calcium and fluorouracil as second-line therapy in treating patients with metastatic colorectal cancer.

PURPOSE: This randomized phase II trial is studying liposome-encapsulated irinotecan hydrochloride PEP02 given together with leucovorin calcium and fluorouracil to see how well it works compared with giving irinotecan hydrochloride together with leucovorin calcium and fluorouracil as second-line therapy in treating patients with metastatic colorectal cancer.

Detailed description

OBJECTIVES:
Primary
* To evaluate the objective response rates (complete response and partial response) in patients with metastatic colorectal cancer treated with liposome-encapsulated irinotecan hydrochloride PEP02, leucovorin calcium, and fluorouracil (FUPEP) Versus irinotecan hydrochloride, leucovorin calcium, and fluorouracil (FOLFIRI 1) or leucovorin calcium, fluorouracil, and irinotecan hydrochloride-modified (FOLFIRI 3-modified).

Secondary
* To determine the safety of these regimens in these patients.
* To determine progression-free survival of these patients.
* To determine overall survival of these patients.

* To assess the quality of life of these patients.
* To assess the correlation of UGT1A family polymorphism and the toxicity of liposome-encapsulated irinotecan hydrochloride PEP02 or irinotecan hydrochloride.

OUTLINE: This is a multicenter study. Patients are stratified, in terms of prognosis, according to treatment center, prognostic score (ECOG performance status [PS] 0 and normal LDH value vs ECOG PS > 1 and/or LDH > 1 times upper limit of normal), and time to progression after first-line therapy (≥ 9 months vs < 9 months). Patients are randomized to 1 of 2 treatment arms.

* Arm I: Patients are assigned to either the FOLFIRI 1 or Modified FOLFIRI 3 treatment groups according to the investigator's discretion.

- FOLFIRI 1: Patients receive irinotecan hydrochloride over 1 hour and leucovorin calcium IV over 2 hours on day 1 and a bolus of fluorouracil followed by fluorouracil IV over 46 hours beginning on day 1. Courses repeat every 14 days in the absence of disease progression or unacceptable toxicity
- Modified FOLFIRI 3: Patients receive irinotecan hydrochloride, leucovorin calcium, and fluorouracil as in FOLFIRI 1. Patients also receive irinotecan hydrochloride IV over 1 hour on day 3. Courses repeat every 14 days in the absence of disease progression or unacceptable toxicity.

* Arm II (FUPEP): Patients receive liposome-encapsulated irinotecan hydrochloride PEP02 IV over 60-90 minutes and leucovorin calcium IV over 2 hours on day 1 and fluorouracil IV over 46 hours beginning on day 1. Courses repeat every 14 days in the absence of disease progression or unacceptable toxicity.

Blood samples are collected periodically for pharmacogenetic analysis of UGT1A family polymorphisms. Quality of life is assessed by using a generic scale EQ-5D and the QLQ-C30 questionnaire at baseline and after courses 4 and 8.

After completion of study treatment, patients are followed up at day 30 and then every 2-3 months thereafter.

Phase	Phase 2
Study type	Interventional
Study design	Treatment
Study design	Randomized
Study design	Open Label
Primary outcome	Measure: Tumor response, in terms of objective response rates (complete response and partial response) Safety Issue? No
Secondary outcome	Measure: Safety Safety Issue? Yes
Secondary outcome	Measure: Progression-free survival Safety Issue? No
Secondary outcome	

	Measure: Overall survival Safety Issue? No
Secondary outcome	Measure: Quality of life Safety Issue? No
Secondary outcome	Measure: Correlation of UGT1A family polymorphism and the toxicity of liposome-encapsulated irinotecan hydrochloride PEP02 or irinotecan hydrochloride Safety Issue? No
Enrollment	88 (Anticipated)
Condition	Colorectal Cancer
Intervention	Drug: FOLFIRI regimen
Intervention	Drug: fluorouracil
Intervention	Drug: irinotecan hydrochloride
Intervention	Drug: leucovorin calcium
Intervention	Drug: liposome-encapsulated irinotecan hydrochloride PEP02
Intervention	Genetic: polymorphism analysis
Intervention	Other: pharmacogenomic studies
Intervention	Procedure/Surgery: quality-of-life assessment
URL	http://cancer.gov/clinicaltrials/FRE-GERCOR-PEPCOL-C10-1
See also	Clinical trial summary from the National Cancer Institute's PDQ® database

Recruitment Information

Status	Recruiting
Start date	2011-05
Primary completion date	2012-12 (Anticipated)

Criteria

DISEASE CHARACTERISTICS:
* Histologically proven adenocarcinoma of colon or rectum

- Metastatic disease, exclusive of bone metastasis
- Not suitable for complete carcinological surgical resection

* Any KRAS status allowed (wild type or mutated)
* Measurable lesion (≥ 1 cm) as assessed by CT scan or MRI according to RECIST criteria (version 1.1)
* Must have received prior oxaliplatin-based chemotherapy for metastatic

disease
* No symptomatic ascites or pleural effusion not evacuated prior to study entry
* No history or evidence of CNS metastasis

PATIENT CHARACTERISTICS:
* WHO or ECOG performance status 0-2
* Absolute neutrophil count ≥ 1,500/mm^3
* Platelet count ≥ 100,000/µL
* Hemoglobin ≥ 9 g/dL (may be transfused to maintain or exceed this level)
* Serum creatinine < 150 µmol/L
* Calculated creatinine clearance > 30 mL/min
* Total bilirubin < 1.5 times upper limit of normal
* Negative serum pregnancy test
* Not pregnant or nursing
* Fertile patients must use effective contraception
* No severe arterial thromboembolic events within the past 6 months, including myocardial infarction and stroke
* No baseline diarrhea > grade 1
* No total or partial bowel obstruction
* No uncontrolled hypercalcemia
* No other prior or concurrent malignancy, except adequately treated in situ carcinoma of the uterine cervix, basal cell or squamous cell carcinoma of the skin, or cancer in complete remission for ≥ 5 years
* No other serious and uncontrolled non-malignant disease
* No known allergy to any excipients of study drugs
* Must be registered in a national health care system (CMU included)

PRIOR CONCURRENT THERAPY:
* See Disease Characteristics
* Prior anti-EGFR therapy allowed
* No prior irinotecan hydrochloride
* No concurrent agents known to have anticancer activity
* No concurrent radiotherapy
* No participation in another clinical trial with any investigational drug or treatments concurrently or within the past 30 days

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

Administrative Data

Organization name	National Cancer Institute (NCI)
Organization study ID	CDR0000701454
Secondary ID	FRE-GERCOR-PEPCOL-C10-1
Secondary ID	EU-21115
Secondary ID	EUDRACT-2010-020468-39
Secondary ID	PHARMAENGINE-FRE-GERCOR-PEPCOL

Sponsor	Groupe Cooperateur Multidisciplinaire en Oncologie (GERCOR)

Liposome-encapsulated Irinotecan Hydrochloride PEP02 or Irinotecan Hydrochloride, Leucovorin Calcium, and Fluorouracil as Second-Line Therapy in Treating Patients With Metastatic Colorectal Cancer (PEPCOL)

This study has been terminated.

(efficacy interim analysis as per protocol)

Sponsor:

Groupe Cooperateur Multidisciplinaire en Oncologie (GERCOR)

ClinicalTrials.gov Identifier:

NCT01375816

First Posted: June 17, 2011

Last Update Posted: June 4, 2015

Information provided by (Responsible Party):

Groupe Cooperateur Multidisciplinaire en Oncologie (GERCOR)

Full Text View | Tabular View | No Study Results Posted | Disclaimer

How to Read a Study Record

Purpose

RATIONALE: Drugs used in chemotherapy, such as liposome-encapsulated irinotecan hydrochloride PEP02, irinotecan hydrochloride, leucovorin calcium, and fluorouracil, work in different ways to stop the growth of tumor cells, either by killing the cells or by stopping them from dividing. It is not yet known whether giving liposome-encapsulated irinotecan hydrochloride PEP02 together with leucovorin calcium and fluorouracil is more effective than giving irinotecan hydrochloride together with leucovorin calcium and fluorouracil as second-line therapy in treating patients with metastatic colorectal cancer.

PURPOSE: This randomized phase II trial is studying liposome-encapsulated irinotecan hydrochloride PEP02 given together with leucovorin calcium and fluorouracil to see how well it

works compared with giving irinotecan hydrochloride together with leucovorin calcium and fluorouracil as second-line therapy in treating patients with metastatic colorectal cancer.

Condition	Intervention	Phase
Colorectal Cancer	Drug: FOLFIRI 1-Bevacizumab Drug: fluorouracil Drug: irinotecan hydrochloride Drug: leucovorin calcium Drug: liposome-encapsulated irinotecan hydrochloride PEP02 Drug: Bevacizumab	Phase 2

Study Type: Interventional

Study Design: Allocation: Randomized
Intervention Model: Parallel Assignment
Masking: None (Open Label)
Primary Purpose: Treatment

Official Title: A Randomized Phase II Study of PEP02 or Irinotecan in Combination With Leucovorin and 5-Fluorouracil in Second Line Therapy of Metastatic Colorectal Cancer

Resource links provided by NLM:

MedlinePlus related topics: Colorectal Cancer

Drug Information available for: Fluorouracil Leucovorin calcium Irinotecan Irinotecan hydrochloride

U.S. FDA Resources

Further study details as provided by Groupe Cooperateur Multidisciplinaire en Oncologie (GERCOR):

Primary Outcome Measures:

- Tumor response [Time Frame: at 2 months]

Assessment of tumor response at 2 month after randomization by RECIST 1.1

Secondary Outcome Measures:

- Safety [Time Frame: before each 2-weeks cycles]

  assessment of adverse events and toxicity according NCI CTC v4.0

- Progression-free survival [Time Frame: the time from the date of randomization to the date of progressive disease (RECIST criteria) or death (any cause)]
- Overall survival [Time Frame: from the date of randomization to the date of patient death, due to any cause, or to the last date the patient was known to be alive]
- Quality of life [Time Frame: at baseline, cycle 4, and cycle 8]

  Quality of life will be assessed by using a generic scale EQ-5D and the QLQ-C30 questionnaire

- Correlation of UGT1A family polymorphism and the toxicity of liposome-encapsulated irinotecan hydrochloride PEP02 or irinotecan hydrochloride [Time Frame: at baseline]

Enrollment:	55
Study Start Date:	May 2011
Study Completion Date:	December 2014
Primary Completion Date:	June 2014 (Final data collection date for primary outcome measure)

Arms	Assigned Interventions
Active Comparator: FOLFIRI 1 or m FOLFIRI3-Bevacizumab FOLFIRI 1-Bevacizumab: Day 1 H0 : Bevacizumab 5 mg/kg, 30-90 min infusion H+1: Irinotecan 180 mg/m² in 250 ml NaCl 0.9%, 1h infusion Folinic Acid 400 mg/m² (l + d racemic form, or l form 200 mg/m²) over 2h H + 3: 5-FU bolus 400 mg/m², 15 min infusion H + 3.5: 5-FU continuous infusion 2400 mg/m² 46-h infusion End of cycle: day 14 modified FOLFIRI3-Bevacizumab H0 :Bevacizumab 5 mg/kg, 30-90 min infusion H+1:Irinotecan 90 mg/m² in 250 ml NaCl	Drug: FOLFIRI 1-Bevacizumab Drug: fluorouracil Drug: irinotecan hydrochloride Drug: leucovorin calcium Drug: Bevacizumab

0.9%, 1h infusion H+1: Folinic Acid 400 mg/m² (l + d racemic form, or l form 200 mg/m²) 2-h infusion H + 3: 5-FU continuous infusion 2400 mg/m² 46-h infusion Day 3 (H+49) H0 Irinotecan 90 mg/m² in 250 ml NaCl 0.9%, 1h infusion End of cycle: day 14	
Experimental: FUPEP-Bevacizumab Day 1 H0 :Bevacizumab 5 mg/kg, 30-90 min infusion H +1 :PEP02 80 mg/m² , 1h30 infusion. The infusion time could be reduced to 1h from cycle 2 if no acute infusion reaction has occured in cycle 1. H +1 : Folinic Acid 400 mg/m² (l + d racemic form, or l form 200 mg/m²) , 2-h infusion H +3 : 5-FU continuous infusion 2400 mg/m² 46-h infusion End of cycle: day 14	Drug: fluorouracil Drug: leucovorin calcium Drug: liposome-encapsulated irinotecan hydrochloride PEP02 Drug: Bevacizumab

Detailed Description:

OBJECTIVES:

Primary

- To evaluate the objective response rates (complete response and partial response) in patients with metastatic colorectal cancer treated with liposome-encapsulated irinotecan hydrochloride PEP02, leucovorin calcium, and fluorouracil (FUPEP) Versus irinotecan hydrochloride, leucovorin calcium, and fluorouracil (FOLFIRI 1) or leucovorin calcium, fluorouracil, and irinotecan hydrochloride-modified (FOLFIRI 3-modified).

Secondary

- To determine the safety of these regimens in these patients.
- To determine progression-free survival of these patients.
- To determine overall survival of these patients.
- To assess the quality of life of these patients.
- To assess the correlation of UGT1A family polymorphism and the toxicity of liposome-encapsulated irinotecan hydrochloride PEP02 or irinotecan hydrochloride.

OUTLINE: This is a multicenter study. Patients are stratified, in terms of prognosis, according to treatment center, prognostic score (ECOG performance status [PS] 0 and normal LDH value vs ECOG PS > 1 and/or LDH > 1 times upper limit of normal), and time to progression after first-line therapy (≥ 9 months vs < 9 months). Patients are randomized to 1 of 2 treatment arms.

- Arm I: Patients are assigned to either the FOLFIRI 1 or Modified FOLFIRI 3 treatment groups according to the investigator's discretion in combination with bevacizumab
  - FOLFIRI 1 in combination with bevacizumab: Patients receive bevacizumab over 30-90 minutes,irinotecan hydrochloride over 1 hour and leucovorin calcium IV over 2 hours on day 1 and a bolus of fluorouracil followed by fluorouracil IV over 46 hours beginning on day 1. Courses repeat every 14 days in the absence of disease progression or unacceptable toxicity
  - Modified FOLFIRI 3 in combination with bevacizumab: Patients receive bevacizumab,irinotecan hydrochloride, leucovorin calcium, and fluorouracil as in FOLFIRI 1. Patients also receive irinotecan hydrochloride IV over 1 hour on day 3. Courses repeat every 14 days in the absence of disease progression or unacceptable toxicity.
- Arm II (FUPEP)in combination with bevacizumab: Patients receive bevacizumab over 30-90 minutes liposome-encapsulated irinotecan hydrochloride PEP02 IV over 60-90 minutes and leucovorin calcium IV over 2 hours on day 1 and fluorouracil IV over 46 hours beginning on day 1. Courses repeat every 14 days in the absence of disease progression or unacceptable toxicity.

Blood samples are collected periodically for pharmacogenetic analysis of UGT1A family polymorphisms. Quality of life is assessed by using a generic scale EQ-5D and the QLQ-C30 questionnaire at baseline and after courses 4 and 8.

After completion of study treatment, patients are followed up at day 30 and then every 2-3 months thereafter.

Eligibility

Information from the National Library of Medicine NIH NLM

Choosing to participate in a study is an important personal decision. Talk with your doctor and family members or friends about deciding to join a study. To learn more about this study, you or your doctor may contact the study research staff using the contacts provided below. For general information, Learn About Clinical Studies

Ages Eligible for Study:	18 Years to 75 Years (Adult, Senior)
Sexes Eligible for Study:	All
Accepts Healthy Volunteers:	No

Criteria

DISEASE CHARACTERISTICS

- Histologically proven adenocarcinoma of colon or rectum
  - Metastatic disease, exclusive of bone metastasis
  - Not suitable for complete carcinological surgical resection
- Patients regardless KRAS status (wild type or mutated) or previous anti EGFR treatment or not.
- Measurable lesion (greater than 1 cm) as assessed by CT scan or MRI according to RECIST criteria (version 1.1)
- Must have received prior oxaliplatin-based chemotherapy for metastatic disease
- No symptomatic ascites or pleural effusion not evacuated prior to study entry
- No history or evidence of CNS metastasis

PATIENT CHARACTERISTICS:

- WHO or ECOG performance status 0-2
- Absolute neutrophil count greater than 1500 per mm3
- Platelet count greater than 100 000 per microL
- Hemoglobin greater than 9 g per dL (may be transfused to maintain or exceed this level)
- INR less or equal than 1.5. aPTT less than 1.5 ULN (exemption:patients on full anticoagulation due to VTE must have an in-range INR.
- Serum creatinine less than 150 micromol per L
- Calculated creatinine clearance greater than 30 mL per min
- Total bilirubin less than 1.5 times upper limit of normal
- Proteinuria less than 2 plus (dipstick urinalysis) or less than 1 g per 24 hours.
- Negative serum pregnancy test
- Not pregnant or nursing
- Fertile patients must use effective contraception
- No severe arterial thromboembolic events within the past 6 months, including myocardial infarction and stroke
- No baseline diarrhea greater than grade 1
- No total or partial bowel obstruction
- No uncontrolled hypercalcemia

- No uncontrolled hypertension, or history of hypertensive crisis, or hypertensive encephalopathy
- No other prior or concurrent malignancy, except adequately treated in situ carcinoma of the uterine cervix, basal cell or squamous cell carcinoma of the skin, or cancer in complete remission for more than 5 years
- No other serious and uncontrolled non-malignant disease
- Major surgery or traumatic injury within the last 28 days.
- No known allergy to any excipients of study drugs
- Must be registered in a national health care system (CMU included)

PRIOR CONCURRENT THERAPY:

- See Disease Characteristics
- Prior anti-EGFR therapy allowed
- No prior irinotecan hydrochloride
- No concurrent agents known to have anticancer activity
- No concurrent radiotherapy
- No participation in another clinical trial with any investigational drug or treatments concurrently or within the past 30 days

Contacts and Locations

Information from the National Library of Medicine NIH NLM

To learn more about this study, you or your doctor may contact the study research staff using the contact information provided by the sponsor.

Please refer to this study by its ClinicalTrials.gov identifier (NCT number): ***NCT01375816***

Locations

France

Hopital Saint Antoine
Paris, France, 75012

Sponsors and Collaborators

Groupe Cooperateur Multidisciplinaire en Oncologie (GERCOR)

Investigators

Principal Investigator: Frederique Maindrault-Goebel, MD Hopital Saint Antoine

More Information

Responsible Party:	Groupe Cooperateur Multidisciplinaire en Oncologie (GERCOR)
ClinicalTrials.gov Identifier:	NCT01375816 History of Changes
Other Study ID Numbers:	CDR0000701454
	FRE-GERCOR-PEPCOL-C10-1
	EU-21115
	EUDRACT-2010-020468-39
	PHARMAENGINE-FRE-GERCOR-PEPCOL
First Submitted:	June 16, 2011
First Posted:	June 17, 2011
Last Update Posted:	June 4, 2015
Last Verified:	November 2013

Keywords provided by Groupe Cooperateur Multidisciplinaire en Oncologie (GERCOR):

adenocarcinoma of the colon	adenocarcinoma of the rectum
recurrent colon cancer	recurrent rectal cancer
stage IV colon cancer	stage IV rectal cancer

Additional relevant MeSH terms:

Colorectal Neoplasms	Leucovorin
Intestinal Neoplasms	Calcium, Dietary
Gastrointestinal Neoplasms	Levoleucovorin
Digestive System Neoplasms	Angiogenesis Inhibitors
Neoplasms by Site	Angiogenesis Modulating Agents
Neoplasms	Growth Substances
Digestive System Diseases	Physiological Effects of Drugs
Gastrointestinal Diseases	Growth Inhibitors
Colonic Diseases	Antineoplastic Agents
Intestinal Diseases	Bone Density Conservation Agents
Rectal Diseases	Antineoplastic Agents, Phytogenic

Bevacizumab
Irinotecan
Camptothecin
Fluorouracil

Topoisomerase I Inhibitors
Topoisomerase Inhibitors
Enzyme Inhibitors
Molecular Mechanisms of Pharmacological Action

View of NCT01494506 on 2011_12_16

ClinicalTrials Identifier: NCT01494506
Updated: 2011_12_16

Descriptive Information

Brief title Study of MM-398 Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer

Official title A Randomized, Open Label Phase 3 Study of MM-398 Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer

Brief summary

The study is an open label, randomized phase 3 study of MM-398 versus 5-fluorouracil (5-FU) and leucovorin (also known as folinic acid) in metastatic pancreatic cancer patients who have progressed on prior gemcitabine based therapy.

Detailed description

Phase Phase 3
Study type Interventional
Study design Treatment
Study design Randomized
Study design Open Label
Study design Parallel Assignment
Study design Efficacy Study
Primary outcome Measure: Overall Survival
Time Frame: 24 months
Safety Issue? No
Secondary outcome Measure: Progression Free Survival
Time Frame: 24 months
Safety Issue? No
Secondary outcome Measure: Time to treatment failure
Time Frame: 24 months
Safety Issue? No
Secondary outcome Measure: Objective response rate
Time Frame: 24 months
Safety Issue? No
Enrollment 270 (Anticipated)
Condition Metastatic Pancreatic Cancer

Arm/Group	Arm Label: MM-398 Experimental MM-398 Q3W IV
Arm/Group	Arm Label: 5 Fluorouracil and Leucovorin IV Active Comparator 5 Fluorouracil and Leucovorin IV
Intervention	Drug: MM-398 Arm Label: MM-398 MM-398 120 mg/m2 IV Q3W
Intervention	Drug: 5 Fluorouracil Arm Label: 5 Fluorouracil and Leucovorin IV 5 Fluorouracil 2000 mg/m2 IV for 4 weeks followed by 2 weeks of rest every 6 weeks
Intervention	Drug: Leucovorin Arm Label: 5 Fluorouracil and Leucovorin IV Leucovorin 200 mg/m2 IV for 4 weeks followed by 2 weeks of rest every 6 weeks

Recruitment Information

Status	Recruiting
Start date	2011-11
Last follow-up date	2014-06 (Anticipated)
Primary completion date	2013-12 (Anticipated)

Criteria

Inclusion Criteria:
- Histologically or cytologically confirmed adenocarcinoma of the exocrine pancreas
- Metastatic disease
- Documented disease progression after prior gemcitabine based therapy
- KPS >/= 70
- Adequate bone marrow function
- Adequate hepatic function
- Adequate renal function

Exclusion Criteria:
- Prior irinotecan treatment
- Active CNS metastasis
- Clinically significant GI disorders
- Major surgery or radiotherapy within 4 weeks of enrollment
- Severe arterial thromboembolic events less than 6 months before inclusion
- NYHA Class III or IV congestive heart failure, ventricular arrhythmias or uncontrolled blood pressure
- Active infection or uncontrolled fever

- Pregnant or breast feeding patients

Gender	Both
Minimum age	18 Years
Healthy volunteers	No

Administrative Data

Organization name	Merrimack Pharmaceuticals
Organization study ID	MM-398-07-03-01
Sponsor	Merrimack Pharmaceuticals
Health Authority	United States: Food and Drug Administration

View of NCT01494506 on 2012_08_09

ClinicalTrials Identifier: NCT01494506
Updated: 2012_08_09

Descriptive Information

Brief title
Study of MM-398 With or Without 5-Fluorouracil and Leucovorin, Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer

Official title
A Randomized, Open Label Phase 3 Study of MM-398, With or Without 5-Fluorouracil and Leucovorin, Versus 5 Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer Who Have Failed Prior Gemcitabine-based Therapy

Brief summary

The study is an open label, randomized phase 3 study of MM-398 with or without 5-Fluorouracil (5-FU) and Leucovorin (also known as folinic acid), versus 5-FU and leucovorin in metastatic pancreatic cancer patients who have progressed on prior gemcitabine based therapy.

Detailed description

Phase Phase 3
Study type Interventional
Study design Treatment
Study design Randomized
Study design Open Label
Study design Parallel Assignment
Study design Efficacy Study
Primary outcome Measure: Overall Survival
Time Frame: 24 months
Safety Issue? No
Secondary outcome Measure: Progression Free Survival
Time Frame: 24 months
Safety Issue? No
Secondary outcome Measure: Time to treatment failure
Time Frame: 24 months
Safety Issue? No
Secondary outcome Measure: Objective response rate
Time Frame: 24 months
Safety Issue? No

Enrollment	405 (Anticipated)
Condition	Metastatic Pancreatic Cancer
Arm/Group	Arm Label: MM-398 Experimental MM-398 Q3W IV
Arm/Group	Arm Label: 5 Fluorouracil and Leucovorin IV Active Comparator 5 Fluorouracil and Leucovorin IV
Arm/Group	Arm Label: MM-398, 5-FU and Leucovorin Experimental MM-398, 5-FU and Leucovorin Q2W IV
Intervention	Drug: MM-398 Arm Label: MM-398 Arm A: MM-398 120 mg/m2 IV Q3W Arm C: MM-398 80mg/m2 IV Q2W
Intervention	Drug: 5 Fluorouracil Arm Label: 5 Fluorouracil and Leucovorin IV Arm B: 5 Fluorouracil 2000 mg/m2 IV for 4 weeks followed by 2 weeks of rest every 6 weeks Arm C: 5 Fluorouracil 2400 mg/m2 IV every 2 weeks
Intervention	Drug: Leucovorin Arm Label: 5 Fluorouracil and Leucovorin IV Arm B: Leucovorin 200 mg/m2 IV for 4 weeks followed by 2 weeks of rest every 6 weeks Arm C: Leucovorin 200 mg/m2 IV every 2 weeks

Recruitment Information

Status	Recruiting
Start date	2011-11
Last follow-up date	2014-06 (Anticipated)
Primary completion date	2013-12 (Anticipated)

Criteria

Inclusion Criteria:
- Histologically or cytologically confirmed adenocarcinoma of the exocrine pancreas
- Metastatic disease
- Documented disease progression after prior gemcitabine based therapy
- KPS >/= 70
- Adequate bone marrow function

- Adequate hepatic function
- Adequate renal function

Exclusion Criteria:
- Active CNS metastasis
- Clinically significant GI disorders
- Severe arterial thromboembolic events less than 6 months before inclusion
- NYHA Class III or IV congestive heart failure, ventricular arrhythmias or uncontrolled blood pressure
- Active infection or uncontrolled fever
- Pregnant or breast feeding patients

Gender	Both
Minimum age	18 Years
Healthy volunteers	No

Administrative Data

Organization name	Merrimack Pharmaceuticals
Organization study ID	MM-398-07-03-01
Sponsor	Merrimack Pharmaceuticals
Health Authority	United States: Food and Drug Administration

Trial record 2 of 9 for: pep02
Previous Study | Return to List | Next Study

A Study of PEP02 in Combination With 5-fluorouracil (5-FU) and Leucovorin (LV) in Advanced Solid Tumors

This study has been completed.

Sponsor:
PharmaEngine

Information provided by (Responsible Party):
PharmaEngine

ClinicalTrials.gov Identifier:
NCT02884128

First received: August 12, 2016
Last updated: August 25, 2016
Last verified: August 2016
History of Changes

Full Text View | Tabular View | No Study Results Posted | Disclaimer | How to Read a Study Record

Purpose

This trial is a multi-center, open-label, phase I, dose escalation study of **PEP02** (liposomal encapsulated irinotecan) in combination with 5-FU and LV in patients with advanced solid tumors.

Condition	Intervention	Phase
Solid Tumor	Drug: **PEP02** Drug: 5-FU Drug: LV	Phase 1

Study Type: Interventional
Study Design: Intervention Model: Single Group Assignment
Masking: Open Label
Primary Purpose: Treatment

Official Title: A Multi-Center, Open-Label Phase I Dose-Escalation Study of **PEP02** in Combination With 5-fluorouracil (5-FU) and Leucovorin (LV) in Advanced Solid Tumors

Resource links provided by NLM:

MedlinePlus related topics: Cancer

Drug information available for: Fluorouracil

U.S. FDA Resources

Further study details as provided by PharmaEngine:

Primary Outcome Measures:

- Dose limiting toxicity (DLT) according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Version 4.0 [Time Frame: 3 weeks] [Designated as safety issue: Yes]

Secondary Outcome Measures:

- objective tumor response according to Response Evaluation Criteria In Solid Tumours (RECIST) 1.0 [Time Frame: 6 weeks] [Designated as safety issue: No]

Enrollment: 16
Study Start Date: January 2006
Study Completion Date: July 2010

Primary Completion Date: August 2008 (Final data collection date for primary outcome measure)

Arms	Assigned Interventions
Experimental: **PEP02** + 5-FU/LV The initial starting dose of **PEP02** is 60 mg/m2, and was escalated by increments of 20 mg/m2 between dose levels. 5-FU and LV were given as 24-hour infusion via an implanted central venous catheter, with dose fixed at 2000 mg/m2 and 200 mg/m2, respectively. **PEP02** was administered on Day 1; 5-FU/LV was started after the end of **PEP02** infusion on Day 1 and also on Day 8.	Drug: **PEP02** **PEP02** was administered on Day 1. Treatment repeated every 3 weeks and it was regarded as one cycle of treatment. Other Name: liposomal irinotecan Drug: 5-FU 5-FU/LV were started after the end of **PEP02** infusion on Day 1 and also on Day 8. Treatment repeated every 3 weeks and it was regarded as one cycle of treatment. Other Name: Fluorouracil Drug: LV 5-FU/LV were started after the end of **PEP02** infusion on Day 1 and also on Day 8. Treatment repeated every 3 weeks and it was regarded as one cycle of treatment. Other Name: Leucovorin

Detailed Description:
In this study, the initial starting dose of PEP02 is 60 mg/m2, and was escalated by increments of 20 mg/m2 between dose levels. 5-FU and LV was given as 24-hour infusion via an implanted central venous catheter, with dose fixed at 2000 mg/m2 and 200 mg/m2, respectively. PEP02 was administered on Day 1; 5-FU/LV was started after the end of PEP02 infusion on Day 1 and also on Day 8. Treatment repeated every 3 weeks and it was regarded as one cycle of treatment.

Eligibility

Ages Eligible for Study: 20 Years to 70 Years (Adult, Senior)
Genders Eligible for Study: Both
Accepts Healthy Volunteers: No

Criteria

Inclusion Criteria:

- Histologically or cytologically confirmed solid tumor which was locally advanced or metastatic and had failed to standard chemotherapy or no standard treatment was available
- ECOG performance status 0 or 1
- With normal organ and marrow function

Exclusion Criteria:

- Have had major surgery, chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or have not recovered from toxicities due to previous treatment
- With known or suspicious primary or secondary brain tumors
- History of allergic reactions attributed to compounds of similar chemical or biologic composition to PEP02, 5-FU or leucovorin
- HBsAg+ or anti-HCV+ patients with splenomegaly (defined as spleen size > 11 cm in CT scan)
- Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, and history of symptomatic congestive heart failure of Functional Class II or more (New York Heart Association) and ischemic heart diseases (i.e. myocardial infarction or angina pectoris), cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements
- Pregnant or breast feeding females (a pregnancy test must be performed on all females who are of child-bearing potential before entering the study and the result must be negative)
- Had received irinotecan treatment

Contacts and Locations

Choosing to participate in a study is an important personal decision. Talk with your doctor and family members or friends about deciding to join a study. To learn more about this study, you or your doctor may contact the study research staff using the Contacts provided below. For general information, see Learn About Clinical Studies.

No Contacts or Locations Provided

More Information

Responsible Party:	PharmaEngine
ClinicalTrials.gov Identifier:	NCT02884128 History of Changes
Other Study ID Numbers:	PEP0203
Study First Received:	August 12, 2016
Last Updated:	August 25, 2016
Health Authority:	Taiwan: Department of Health
Individual Participant Data Plan to Share IPD:	No

Additional relevant MeSH terms:

Fluorouracil
Antimetabolites
Molecular Mechanisms of Pharmacological Action
Antimetabolites, Antineoplastic
Antineoplastic Agents
Immunosuppressive Agents
Immunologic Factors
Physiological Effects of Drugs

ClinicalTrials.gov processed this record on November 30, 2016

A Study of PEP02 in Combination With 5-fluorouracil (5-FU) and Leucovorin (LV) in Advanced Solid Tumors

This study has been completed.

Sponsor:
PharmaEngine

ClinicalTrials.gov Identifier:
NCT02884128

First Posted: August 30, 2016
Last Update Posted: August 30, 2016

Information provided by (Responsible Party):
PharmaEngine

Full Text View | Tabular View | No Study Results Posted | Disclaimer
How to Read a Study Record

Purpose

This trial is a multi-center, open-label, phase I, dose escalation study of PEP02 (liposomal encapsulated irinotecan) in combination with 5-FU and LV in patients with advanced solid tumors.

Condition	Intervention	Phase

Solid Tumor	Drug: PEP02 Drug: 5-FU Drug: LV	Phase 1

Study Type: Interventional

Study Design: Intervention Model: Single Group Assignment
Masking: None (Open Label)
Primary Purpose: Treatment

Official Title: A Multi-Center, Open-Label Phase I Dose-Escalation Study of PEP02 in Combination With 5-fluorouracil (5-FU) and Leucovorin (LV) in Advanced Solid Tumors

Resource links provided by NLM:

Drug Information available for: Fluorouracil

U.S. FDA Resources

Further study details as provided by PharmaEngine:

Primary Outcome Measures:

- Dose limiting toxicity (DLT) according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Version 4.0 [Time Frame: 3 weeks]

Secondary Outcome Measures:

- objective tumor response according to Response Evaluation Criteria In Solid Tumours (RECIST) 1.0 [Time Frame: 6 weeks]

Enrollment: 16
Study Start Date: January 2006
Study Completion Date: July 2010
Primary Completion Date: August 2008 (Final data collection date for primary outcome measure)

Arms	Assigned Interventions

Experimental: PEP02 + 5-FU/LV The initial starting dose of PEP02 is 60 mg/m2, and was escalated by increments of 20 mg/m2 between dose levels. 5-FU and LV were given as 24-hour infusion via an implanted central venous catheter, with dose fixed at 2000 mg/m2 and 200 mg/m2, respectively. PEP02 was administered on Day 1; 5-FU/LV was started after the end of PEP02 infusion on Day 1 and also on Day 8.	Drug: PEP02 PEP02 was administered on Day 1. Treatment repeated every 3 weeks and it was regarded as one cycle of treatment. Other Name: liposomal irinotecan Drug: 5-FU 5-FU/LV were started after the end of PEP02 infusion on Day 1 and also on Day 8. Treatment repeated every 3 weeks and it was regarded as one cycle of treatment. Other Name: Fluorouracil Drug: LV 5-FU/LV were started after the end of PEP02 infusion on Day 1 and also on Day 8. Treatment repeated every 3 weeks and it was regarded as one cycle of treatment. Other Name: Leucovorin

Detailed Description:

In this study, the initial starting dose of PEP02 is 60 mg/m2, and was escalated by increments of 20 mg/m2 between dose levels. 5-FU and LV was given as 24-hour infusion via an implanted central venous catheter, with dose fixed at 2000 mg/m2 and 200 mg/m2, respectively. PEP02 was administered on Day 1; 5-FU/LV was started after the end of PEP02 infusion on Day 1 and also on Day 8. Treatment repeated every 3 weeks and it was regarded as one cycle of treatment.

Eligibility

Information from the National Library of Medicine NIH NLM

Choosing to participate in a study is an important personal decision. Talk with your doctor and family members or friends about deciding to join a study. To learn more about this study, you or your doctor may contact the study research staff using the contacts provided below. For general information, Learn About Clinical Studies.

Ages Eligible for Study:	20 Years to 70 Years (Adult, Senior)
Sexes Eligible for Study:	All
Accepts Healthy Volunteers:	No

Criteria

Inclusion Criteria:

- Histologically or cytologically confirmed solid tumor which was locally advanced or metastatic and had failed to standard chemotherapy or no standard treatment was available
- ECOG performance status 0 or 1
- With normal organ and marrow function

Exclusion Criteria:

- Have had major surgery, chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or have not recovered from toxicities due to previous treatment
- With known or suspicious primary or secondary brain tumors
- History of allergic reactions attributed to compounds of similar chemical or biologic composition to PEP02, 5-FU or leucovorin
- HBsAg+ or anti-HCV+ patients with splenomegaly (defined as spleen size > 11 cm in CT scan)
- Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, and history of symptomatic congestive heart failure of Functional Class II or more (New York Heart Association) and ischemic heart diseases (i.e. myocardial infarction or angina pectoris), cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements
- Pregnant or breast feeding females (a pregnancy test must be performed on all females who are of child-bearing potential before entering the study and the result must be negative)
- Had received irinotecan treatment

Contacts and Locations

No Contacts or Locations Provided

More Information

Publications automatically indexed to this study by ClinicalTrials.gov Identifier (NCT Number):

Chiang NJ, Chao TY, Hsieh RK, Wang CH, Wang YW, Yeh CG, Chen LT. A phase I dose-escalation study of PEP02 (irinotecan liposome injection) in combination with 5-fluorouracil and leucovorin in advanced solid tumors. BMC Cancer. 2016 Nov 21;16(1):907.

Responsible Party:	PharmaEngine
ClinicalTrials.gov Identifier:	NCT02884128 History of Changes
Other Study ID Numbers:	PEP0203
First Submitted:	August 12, 2016
First Posted:	August 30, 2016
Last Update Posted:	August 30, 2016
Last Verified:	August 2016

Individual Participant Data (IPD) Sharing Statement:

Plan to Share IPD: No

Additional relevant MeSH terms:

Fluorouracil
Antimetabolites
Molecular Mechanisms of Pharmacological Action
Antimetabolites, Antineoplastic
Antineoplastic Agents
Immunosuppressive Agents
Immunologic Factors
Physiological Effects of Drugs

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journal homepage: www.elsevier.com/locate/jconrel

Review

State-of-the-art in design rules for drug delivery platforms: Lessons learned from FDA-approved nanomedicines

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Tumor targeting
Active targeting
Nanoparticles
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Enhanced permeability and retention (EPR) effect

ABSTRACT

The ability to efficiently deliver a drug to a tumor site is dependent on a wide range of physiologically imposed design constraints. Nanotechnology provides the possibility of creating delivery vehicles where these design constraints can be decoupled, allowing new approaches for reducing the unwanted side effects of systemic delivery, increasing targeting efficiency and efficacy. Here we review the design strategies of the two FDA-approved antibody–drug conjugates (Brentuximab vedotin and Trastuzumab emtansine) and the four FDA-approved nanoparticle-based drug delivery platforms (Doxil, DaunoXome, Marqibo, and Abraxane) in the context of the challenges associated with systemic targeted delivery of a drug to a solid tumor. The lessons learned from these nanomedicines provide an important insight into the key challenges associated with the development of new platforms for systemic delivery of anti-cancer drugs.

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1. Introduction

Local delivery of a drug to a solid tumor has the potential to overcome many of the unwanted side effects of systemic delivery, however, the design of nanoparticle-based platforms for local delivery is extremely challenging and must overcome a number of physiologically imposed design constraints (Fig. 1). Research on nanomedicines for *in vivo* diagnostics and/or therapeutics has increased dramatically over the past 10 years, and yet there are only two FDA-approved antibody–drug conjugates (Brentuximab vedotin and Trastuzumab emtansine) and four FDA-approved nanoparticle-based drug delivery platforms (Doxil, DaunoXome, Marqibo, and Abraxane) (Table 1). Here we review the design of these FDA-approved therapeutic platforms in the context of the challenges associated with systemic targeted delivery of a drug to a solid tumor.

Antibody–drug conjugates (ADCs) are a conceptually simple approach to target a drug to a tumor and reduce the toxic side effects associated with systemic delivery of a free drug. However, meeting the design criteria for ADCs has proven to be challenging. While numerous strategies for targeted drug delivery and combined theranostic nanoparticle platforms have been proposed, there have been very few systematic studies that could ultimately provide design rules for the development of new platforms. In the research community, more weight is often given to new nanoparticle drug delivery platforms, regardless of the potential for clinical translation. The unglamorous research required to fully characterize the components of a system or to contribute to the development of design rules has been largely overlooked. The quest for increasingly complex nanoparticle platforms often ignores the difficulties in overcoming the physiologically imposed constraints in accumulating a drug at therapeutic concentrations in a tumor while avoiding toxic side effects in normal tissue, the chief function of nanoparticle-based delivery.

In this review we summarize the design rationale for the six current FDA-approved nanomedicines: ADCs, liposome-based delivery platforms, and albumin-bound nanoparticles. We focus on the lessons learned from the design of these platforms in the context of the pharmacokinetics and the physiologically imposed design constraints, including circulation, the mononuclear phagocyte system (MPS), the enhanced permeability and retention (EPR) effect, tumor transport, and toxicity. Finally, we summarize the current status of design rules for nanoparticle drug delivery platforms based on these six FDA-approved nanomedicines.

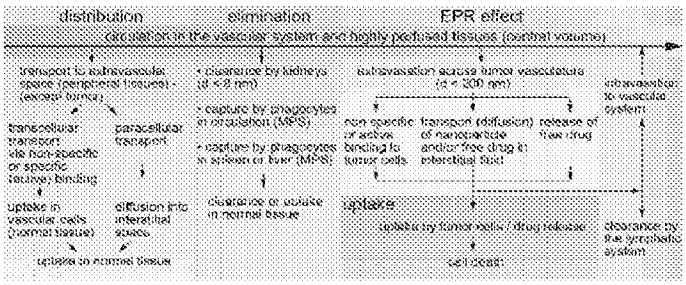

Fig. 1. Schematic illustration of physiologically imposed design constraints for nanoparticle-based targeted drug delivery. After systemic delivery of a nanoparticle-based platform, distribution in peripheral tissues (except the tumor) can lead to uptake in normal tissues and the potential for adverse side effects. Nanoparticles can be targeted by the mononuclear phagocyte system (MPS) in circulation or in the liver and spleen. Small nanoparticles or nanoparticle fragments can be cleared by the kidneys. The enhanced permeability and retention (EPR) effect is usually the key to accumulation of a nanoparticle-platform in a solid tumor. A nanoparticle may be taken up by active targeting of cell surface biomarkers on the tumor cells or by passive non-specific binding. Drug release after uptake may be by passive diffusion (e.g. from a polymer nanoparticle) or by exploiting a cleavable linker. Transport of the nanoparticle platform or free drug to the tumor core usually relies on passive diffusion in the interstitial space.

2. Chemotherapy vs targeted therapy

In the treatment of cancer, the use of one or more cytotoxic small molecules is widely used to kill highly proliferative cancer cells. However, these drugs also kill other proliferative cells in bone marrow, the gastrointestinal tract (stomach and intestines), and hair follicles, leading to common side effects such as compromised immune system (due to decreased production of leukocytes, red blood cells, and platelets), inflammation and ulceration of mucous membranes in the GI tract, and hair loss. Small molecule chemotherapeutics generally include: alkylating agents (e.g. cisplatin), anti-metabolites (e.g. gemcitabine), anti-microtubule agents (e.g. paclitaxel, vincristine), topoisomerase inhibitors (e.g. topotecan), and cytotoxic inhibitors (e.g. doxorubicin).

Targeted antibody therapies reduce the toxic side effects of anticancer drugs in normal cells and tissues by targeting a cell-surface receptor that will either directly or indirectly kill cancer cells. Indirect strategies include inducing an immune response that leads to cancer cell apoptosis or inhibiting angiogenesis [1–5]. Common targets for anticancer antibodies are the B-lymphocyte antigen (CD20) expressed by lymphomas and some leukemias, vascular endothelial growth factor receptor (VEGFR) expressed by vascular endothelial cells involved in angiogenesis, and one of the epidermal growth factor receptors (e.g. HER2) upregulated in some cancer cells [5]. Examples of FDA approved antibodies for cancer therapy include rituximab, trastuzumab, and bevacizumab.

The large libraries of cell surface markers overexpressed in cancer cells have provided a resource in identifying potential candidates for targeted drug delivery. However, expression levels are relative to normal cells — many of these markers are also expressed by normal endothelial cells but at lower levels. For example, two common receptors for targeting: the transferrin receptor (TfR1) and the folate receptor (FR-α) are overexpressed in many tumors but are also expressed at low levels in many normal tissues [6,7]. Consequently, efficient targeting of a cell surface marker may result in delivery of a nanoparticle to both tumor and normal cells. Furthermore, a systemically delivered nanoparticle platform will be exposed to more normal cells than tumor cells during circulation.

Nanoparticle-based platforms combining a drug, biological product, and/or device (e.g. nanoparticle), are considered combination products. The path for translating new combination drug therapies is complex and the roadmap for commercialization is not well-defined. Preclinical development of a new molecular entity (NME, i.e. drug) requires assessment of Chemistry, Manufacturing, and Controls (CMC), and includes characterization of the product (e.g. physicochemical properties, pharmacokinetics, safety, toxicity, and metabolism) and evaluation of the manufacturing process [8]. While there has been considerable progress, standards for CMC of nanomedicines are not well established. Therefore, platforms that are as simple as possible and use materials with established biocompatibility are more likely to be approved for clinical use.

Table 1
Summary of FDA-approved antibody–drug conjugates (ADCs) and nanomedicines.

Platform	Class	Drug	d (nm)	Drug/carrier ratio	Key design feature(s)	Problem addressed
Brentuximab vedotin	ADC	Monomethyl auristan E	~10	≤8	Valine–citrulline linker cleaved by cathepsin in endosomes	Monomethyl auristan E (MMAE) is too toxic to be used alone
Trastuzumab emtansine	ADC	Mertansine	~10	≤8	Non-cleavable linker; release of drug by proteolytic degradation of antibody in endosomes	Mertansine is too toxic to be used alone
Doxil	Liposome	Doxorubicin	100	10,000–15,000	Lipid encapsulation for high drug/carrier ratio, polyethylene glycol coating to evade MPS, crystallization of drug in liposome minimizes escape during circulation	Drug toxicity and adverse cardiac side effects
DaunoXome	Liposome	Daunorubicin	50	~10,000	No polyethylene glycol coating, targeted by MPS resulting in slow release into circulation	Drug toxicity and adverse cardiac side effects
Marqibo	Liposome	Vincristine	100	~10,000	No polyethylene glycol coating, targeted by MPS resulting in slow release into circulation	Drug toxicity and adverse side effects
Abraxane	Protein carrier	Paclitaxel	130	>10,000	Non-specific binding of paclitaxel to albumin	Overcomes very low solubility of paclitaxel

3. Antibody–drug conjugates (ADCs), liposomes, and albumin-bound nanoparticles

3.1. Antibody–drug conjugates (ADCs)

The use of targeted antibody therapy reduces the side effects associated with potent cytotoxic drugs but is limited to antibodies that can modulate a pathway or process that results in cancer cell apoptosis. The conjugation of antibodies to anticancer drugs overcomes this limitation by separating the design requirements of targeting and treatment: the antibody is used to target a molecule that is overexpressed on cancer cells and the drug induces cell death [1–3,9,10]. The antibody is usually covalently conjugated to the drug with a cleavable (e.g. peptide or disulfide) or non-cleavable (e.g. thioether) linker [11]. The conjugation site on the antibody is usually a surface accessible residue with a reactive group, such as the amine side group on a lysine. Using this approach, several drug molecules (typically up to 8) can be conjugated to a single antibody. A disadvantage to random conjugation is that the linker and/or drug may block the antigen binding sites on the antibody. In addition, many drugs have limited solubility and require the addition of a polyethylene glycol unit to the linker. While this concept appears straightforward, there are currently only two FDA-approved antibody–drug conjugates for cancer therapy: Brentuximab vedotin and Trastuzumab emtansine.

Brentuximab vedotin, approved by the FDA in 2011, targets CD30 overexpressed in lymphomas [10,12,13]. The antibody brentuximab is conjugated to monomethyl auristan E (MMAE), an anti-mitotic drug that is too toxic to be used alone. The drug is conjugated to the thiolated antibody *via* a maleimide linkage, and includes a valine–citrulline peptide sequence that is cleaved by cathepsin, a protease that degrades proteins and is activated by the low pH in lysosomes [10,12,13]. The valine–citrulline linker is stable in serum with only 2% of the drug released after 10 days [14].

Trastuzumab emtansine, approved by the FDA in 2013 for treatment of HER2 + metastatic breast cancer, is an ADC with trastuzumab conjugated to the drug mertansine *via* a non-cleavable linkage [15,16]. The heterobifunctional cross-linker has succinimide ester and maleimide reactive groups at either end of a cyclohexane spacer and covalently couples the sulfhydryl-terminated drug to a surface accessible amine (e.g. lysine residue) on the antibody [17]. The pharmacokinetics of Brentuximab vedotin and Trastuzumab emtansine are similar (Fig. 2): both have moderate AUCs, relatively low clearance, and elimination half-times of 3–4 days [14,15,17,18].

The small number of FDA-approved ADCs highlights the difficulty in coupling an antibody with a drug for cancer therapy. The key design requirements are that the ADC is stable in blood, targets tumor cells, and releases the drug to the appropriate intracellular or paracellular compartment(s) after uptake. Although the overall requirements are well understood, the design of new antibody/drug pairs remains largely empirical. The main technological challenges in developing ADCs are the difficulty in selecting antibody/drug pairs and linkers that result in selective targeting and efficient intratumoral release of the free drug [10,12,13,19,20].

3.1.1. Linkers for drug ADCs and other platforms

For systemically delivered drug conjugates where the drug is covalently coupled to the delivery platform, the drug must be released at the tumor site. At the same time, the linker should be stable in circulation to avoid the cytotoxic side effects of the free drug. For example, the linker should not be degraded by endogenous proteases in the blood. Since ADCs are usually taken up by an endocytic pathway, drug release usually exploits the local biochemistry in endosomes. Linkers are generally divided into two main categories: cleavable and non-cleavable (Fig. 3) [31]. Cleavable linkers generally exploit chemical or enzymatic cleavage and result in direct release of the drug and the remaining linker fragment. For example, the valine–citrulline peptide linkage is cleaved by cathepsin in lysosomes, and is exploited in Brentuximab vedotin. Hydrazone linkers are stable at neutral pH but are unstable in the acidic environment in lysosomes. Disulfide linkages are cleaved by reducing agents such as glutathione (a glutamic acid–cysteine–glycine peptide) that are present at much higher concentrations inside cells than in circulation [32], although the efficiency of disulfide cleavage in endosomes is relatively low [33].

Non-cleavable linkers, such as thioether linkers, are generally more stable in circulation than cleavable linkers. For platforms such as ADCs, non-cleavable linkers rely on intracellular proteases to degrade the antibody and release the drug and linker. The strategy of degrading the delivery platform after uptake is usually not feasible in more complex systems.

3.1.2. Drug release

Anti-cancer drugs used in chemotherapy have different mechanisms of action and hence therapeutic efficacy is dependent on drug release and delivery to the appropriate compartment within a cell [34–39]. For example, anti-microtubule drugs (e.g. paclitaxel) must access microtubules in the cytoplasm whereas alkylating agents (e.g. cisplatin), antimetabolites (e.g. gemcitabine), topoisomerase inhibitors (topotecan), and anti-tumor antibiotics (e.g. doxorubicin) must be trafficked to the nucleus. After binding to the target molecule, ADC–receptor complexes are usually internalized by endocytosis resulting in intracellular trafficking in endosomes [1,12]. Therefore, cleavage of the drug from the antibody and escape from the endosome are critical final steps in drug therapy.

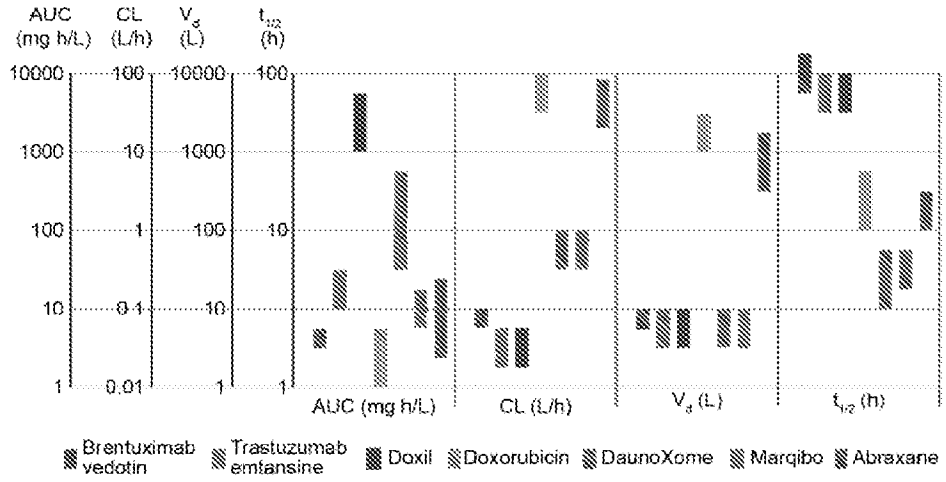

Fig. 2. Summary of pharmacokinetic parameters for FDA-approved antibody–drug conjugates (ADCs) and nanomedicines: Area Under the Curve (AUC), clearance (CL), distribution volume (V_d), and elimination half-time ($t_{1/2}$). Each bar represents the range of mean values obtained from clinical trials in humans. Brentuxumab vedotin [21], Trastuzumab emtansine [18], Doxil [22], Doxorubicin [23–25], DaunoXome [26], Marqibo [27], and Abraxane [28–30]. Doxil has high AUC, low clearance rate, small distribution volume, and a long elimination half-time. These features are largely due to the polyethylene glycol coating that provides extended evasion of the MPS and minimizes distribution into peripheral tissues. DaunoXome and Marqibo also have a small distribution volume but are designed to have faster MPS uptake and shorter elimination half-times by having no polyethylene glycol coating. The ADCs have low clearance rates, small distribution volumes, and long elimination half-time, but relative low AUCs. Abraxane has a relatively fast clearance rate, large distribution volume, and moderate elimination half-time.

3.2. Liposomes

Antibody–drug conjugates typically deliver only a few drug molecules per antibody. Nanoparticle-based platforms represent an alternative approach to reducing the side effects of toxic anti-cancer drugs with very high drug/carrier ratios. There are currently four FDA-approved nanoparticle-based drug therapy platforms: liposome-based and albumin-bound drug conjugates (Table 1).

Liposomes are artificial vesicles with one or more concentric layers of phospholipids and an internal aqueous core. Small unilamellar vesicles (typically 30–100 nm in diameter) are less stable than their larger counterparts (100 nm–1 μm) due to the high curvature and hence high surface tension. The combination of a lipid outer surface and aqueous core allows targeting and stability requirements to be decoupled from drug loading. The aqueous core can be loaded with drugs, DNA, siRNA, and/or contrast agents [40].

Doxil and Myocet are liposomal formulations of doxorubicin, DaunoXome is a liposomal formation of daunorubicin, and Marqibo is a liposomal formulation of vincristine. Of these Doxil, DaunoXome, and Marqibo are FDA-approved. Doxil is sold as Caelyx outside the USA, and Myocet is a non-pegylated liposome-based drug sold outside the USA.

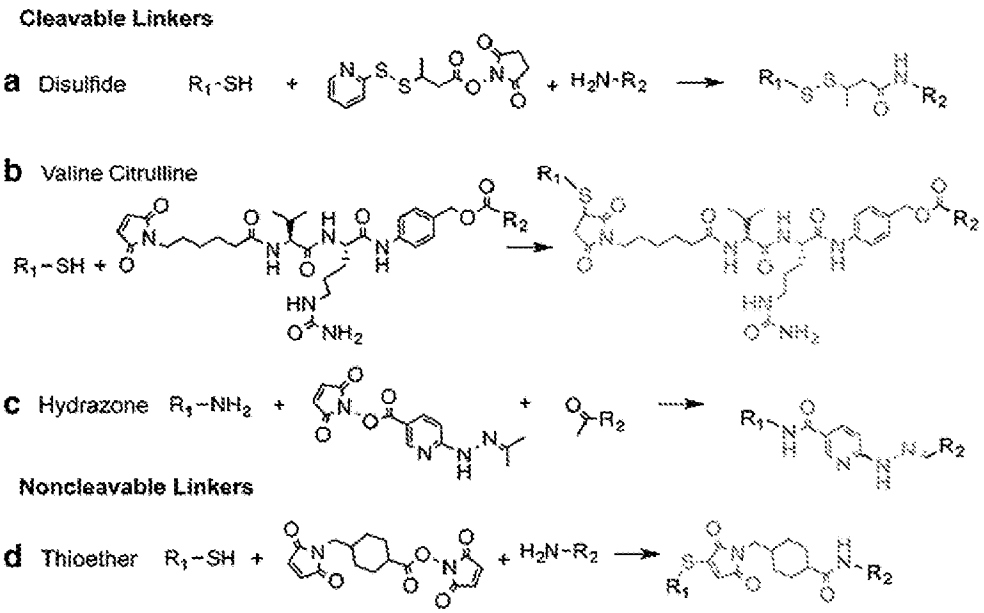

Fig. 3. Common cleavable linkers for ADCs. (a) Disulfide bonds are formed using a cross-linking agent such as N-succinimidyl 3-(2-pyridyldithio)butyrate (SPDP) to link a thiol group to an amine. Disulfide bonds are cleavable under reducing agents. (b) The valine–citrulline bond is a common peptide linkage that can be attached to antibodies *via* accessible thiols. The peptide sequence cleaved by proteases, such as cathepsin, in acidic endosomes or lysosomes. (c) Hydrazone bonds can be formed using succinimidyl 4-hydrazinonicotinate acetone hydrazone (SANH) to link an amine to an aldehyde containing. Hydrazone bonds are stable at neutral pH but can be cleaved in acidic lysosomes. (d) The thiother bond is the most common non-cleavable linker, formed between a maleimide group (often on the drug) and a sulfhydryl group on the antibody using a crosslinking agent such as succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC). In general, drug release is achieved by proteolytic degradation of the targeting antibody. The linker is shown in blue with the bond group in red.

Doxil was FDA-approved for AIDS-related Kaposi's sarcoma in 1995, for ovarian cancer in 1999, and for multiple myeloma in 2007. In 2013 the use of the generic version Lipodox was approved for treatment of ovarian cancer and Kaposi's sarcoma [41]. DaunoXome was approved by the FDA in 1996 for treatment of Kaposi's sarcoma. Marqibo was FDA-approved in 2012 for leukemia. Myocet is sold outside the USA for treatment of breast cancer.

Doxil is formulated from a combination of fully hydrogenated soy phosphatidylcholine (HSPC), cholesterol, and a lipid with a polyethylene glycol (PEG) head group (DSPE-PEG2k) in a mole ratio of 56.4:38.3:5.3. Taking a hydrodynamic diameter of a 2k PEG of about 2 nm [42], the PEG groups correspond to an area coverage of about 60%. The DSPE-PEG provides a polymer coating that can inhibit protein adhesion and prolong evasion of the MPS [43,44]. Such coatings lead to long circulation half-times (3–4 days in humans) and are essential to achieve significant passive accumulation at a tumor site. The Doxil liposomes are about 100 nm in diameter and have 10,000–15,000 doxorubicin molecules per liposome. Drug loading into preformed liposomes is achieved using a base exchange mechanism. The liposomes contain a high concentration of ammonium sulfate resulting in exchange of the drug, which is a weak base, for ammonium ions across the bilayer [45]. The concentration of doxorubicin in Doxil liposomes is about 45 mM, larger than the solubility limit, resulting in precipitation of doxorubicin sulfate crystals in the liposome.

A potential limitation of liposomes is drug leakage and stability in circulation. The presence of cholesterol increases the bilayer cohesiveness and reduces leakage. In addition, the formation of a solid phase minimizes osmotic effects and is thought to contribute to stability, with more than 98% of the circulating drug remaining inside liposomes [22,46–48]. The distribution volume for Doxil is close to the volume of blood indicating that the liposomes exhibit minimal uptake by normal tissues (Fig. 2). The pegylated lipids in the liposomes minimize opsonization and uptake by phagocytes in the MPS system, resulting in elimination half-times of 3–4 days [22,49]. The area under the plasma concentration curve (AUC) for Doxil is large due to the small distribution volume and long elimination half-time. In contrast, the distribution volume for free doxorubicin is very large illustrating that a significant amount of the drug is taken up in normal tissues [23–25,50]. The AUC is for doxorubicin is about three orders of magnitude smaller than Doxil resulting in a clearance rate about three orders of magnitude larger [23–25,50]. The elimination half-time for doxorubicin is about 20–25 h.

Experiments in animal models have shown that pegylated liposomes extravasate from the vascular system and accumulate at the tumor site *via* the EPR effect [22,45]. The mechanism of drug release from the liposomes and uptake by tumor cells is not well understood. In contrast to ADCs, direct uptake of liposomes by tumor cells is thought to be negligible [45]. Possible mechanisms include: disruption of the lipid bilayer by phospholipases, collapse of the ammonium salt gradient or by uptake and release by macrophages at the tumor site [22,45].

DaunoXome, Marqibo, and Myocet liposomes are not pegylated and are designed to be phagocytosed by monocytes in circulation. Allowing the liposomes to be targeted by the MPS avoids high plasma concentrations and provides a reservoir from which the free drug can enter the circulation, similar to a slow infusion. This approach has been exploited for the delivery of antiparasitic or antimicrobial drugs to treat infections localized to the mononuclear phagocytic system [43]. Myocet has a POPC:cholesterol mole ratio of 55.8:44.2 and is about 180 nm in diameter. DaunoXome has a DSPC:cholesterol ratio of 2:1 molar ratio and is about 50 nm in diameter [26]. Marqibo has a sphingomyelin:cholesterol mole ratio of 57.4:42.6 is about 100 nm in diameter [27]. DaunoXome and Marqibo have small distribution volumes indicating relatively small distribution into peripheral tissues, but relatively short elimination half-times less then 10 h highlighting the relatively fast uptake by the MPS (Fig. 2) [26,27,51].

Liposomal drug formations have been approved for a number of indications. In many cases, clinical trials are designed for hard to treat cancers with poor prognosis and dose limiting side effects [27,52–54]. Evaluating the efficacy of these therapies is not straightforward since clinical trials are usually designed to compare the liposomal formulation to the state-of-the-art drug therapy. Consequently, there are few clinical trials comparing a liposomal drug with its corresponding free drug. While many trials for Doxil and DaunoXome demonstrate comparable survival rates to the state-of-the-art drug therapy, the cardiotoxicity associated with free doxorubicin and daunorubicin is significantly reduced [26,45,55,56]. Small improvements in survival rates have been reported for Doxil compared to paclitaxel in the treatment of HIV-associated Kaposi's sarcoma [57] and Doxil compared to topotecan in the treatment of recurrent or non-responsive ovarian cancer [58].

Existing FDA-approved liposome technologies rely on passive accumulation through the EPR effect. Current challenges include developing platforms with improved biodistribution, pharmacokinetic properties, and active targeting. While various active targeting strategies have been explored, there are no FDA-approved platforms [43,59], highlighting the difficulties in reliably improving accumulation at the tumor site with active targeting.

3.3. Albumin-bound nanoparticles

A strategy for delivery of drugs with low aqueous solubility is to take advantage of endogenous proteins. Albumin reversibly binds hydrophobic molecules, such as vitamins and hormones, and is the most abundant protein in plasma. Albumin is a 67 kDa protein, about 4 nm in diameter and 15 nm long, similar in size to an antibody, and has a hydrodynamic radius of about 3.5 nm [60,61]. Abraxane is albumin bound paclitaxel, or nab-paclitaxel (nanoparticle albumin bound), formed from lyophilized human serum albumin and paclitaxel [62]. Due to its very low solubility (<0.01 mg/mL), paclitaxel is usually mixed with the non-ionic solvent Cremophor to form an emulsion in aqueous solution. However, the solvent is toxic and can lead to a wide range of allergic reactions. Non-specific binding of paclitaxel to albumin provides an alternative solution to overcome the low solubility.

In suspension, Abraxane particles are about 130 nm in diameter, however, on injection they dissociate into smaller albumin–paclitaxel complexes or unbound paclitaxel [63]. Albumin mediates endocytosis of plasma components *via* the albondin receptor and hence it has been suggested that albumin–paclitaxel complexes may be taken up by albumin mediated endocytosis. Abraxane was approved by the FDA in 2005 for treatment of breast, lung, pancreatic, and small cell lung cancers [62]. The pharmacokinetics of Abraxane (Fig. 2) and paclitaxel-Cremophor are very similar. Both exhibit an initial distribution phase in peripheral tissues, characterized by a large distribution volume, a moderate AUC, a relatively high clearance, and an elimination half-time of about 1 day (Fig. 2) [28–30].

4. Physiologically imposed design constraints

4.1. Circulation: distribution in the vascular system and peripheral tissues

Systemic delivery through the circulation is the most widely used method for drug delivery (Fig. 1). In humans, vessels range in diameter from 2.5 cm in large arteries to about 7 μm in capillaries, and the volume of blood in an adult is about 4–5 L [64]. Molecules can exit the circulation by binding to vessel walls and highly perfused tissues, transcellular transport, paracellular transport, filtration in the kidneys, the MPS, the EPR effect, or defects in the vasculature, for example due to inflammation or injury. Transcellular transport or transcytosis across the endothelium is also expected to be limited to very small particles, although its role in nanomedicine is not well understood. Paracellular transport at cell–cell adherens junctions in the endothelium is limited

to molecules ≤2 nm in size, although particles as large as 6 nm may be able to cross the endothelium at post-capillary venules. The glomerulus in the kidneys filters particles less than about 60–70 kDa, or about 8 nm. Proteins in the blood can bind to particles creating a protein corona [65–67], and some of these proteins are targets for receptors on phagocytic cells resulting in clearance by the MPS. Tumor neovasculature and sites of inflammation or injury are leaky and can lead to exit from circulation by the EPR effect. Finally, defects in the vasculature due to injury or disease can lead to local tissue accumulation.

Blood contains various proteins, molecules, and ions, along with red blood cells, leukocytes, and platelets. Human serum albumin, the most abundant protein in blood, is responsible for transport of a wide range of molecules in the body. The molecular weight of albumin is 67 kDa, about the threshold for glomerular filtration in the kidneys. Antibodies, complement proteins, and circulating proteins such as mannose-binding lectin are examples of opsonins, a class of proteins that promote uptake by the MPS.

4.2. The mononuclear phagocyte system (MPS)

The MPS consists of phagocytic cells, such as monocytes in blood and macrophages in tissue, which participate in inflammation, infection, and cancer (Fig. 4) [68]. One of the functions of these cells is to remove pathogens and foreign matter from the body. The spleen, the largest unit in the MPS, primarily functions as a filter for blood. Macrophages in the spleen and Kupffer cells in the liver can also sequester nanoparticles resulting in accumulation in these organs.

Phagocytes have receptors for molecules, termed opsonins, which can initiate binding and removal. Nanoparticles that become coated with opsonins are likely to be taken up by phagocytic cells in circulation. Examples of receptors on phagocytic cells include CR1 and Fc receptors. Therefore, an accessible Fc region of an IgG antibody on a nanoparticle platform can initiate removal from circulation by the MPS.

For most nanoparticle platforms, the circulation time should be maximized to maximize accumulation in the tumor by the EPR effect. The most successful strategy developed to date to avoid the MPS is pegylation. Pegylated liposomes (e.g. Doxil) have an elimination half-time of 2–4 days. However, as described previously, other strategies for drug delivery take advantage of fast uptake by the MPS. Non-pegylated liposomes (e.g. Myocet) are taken up very quickly by the MPS, minimizing high plasma concentrations, and then returned to circulation over time simulating sustained delivery.

Polyethylene glycol is widely used to inhibit protein and cell adhesion on surfaces, but it does not prevent adhesion [69]. An alternative

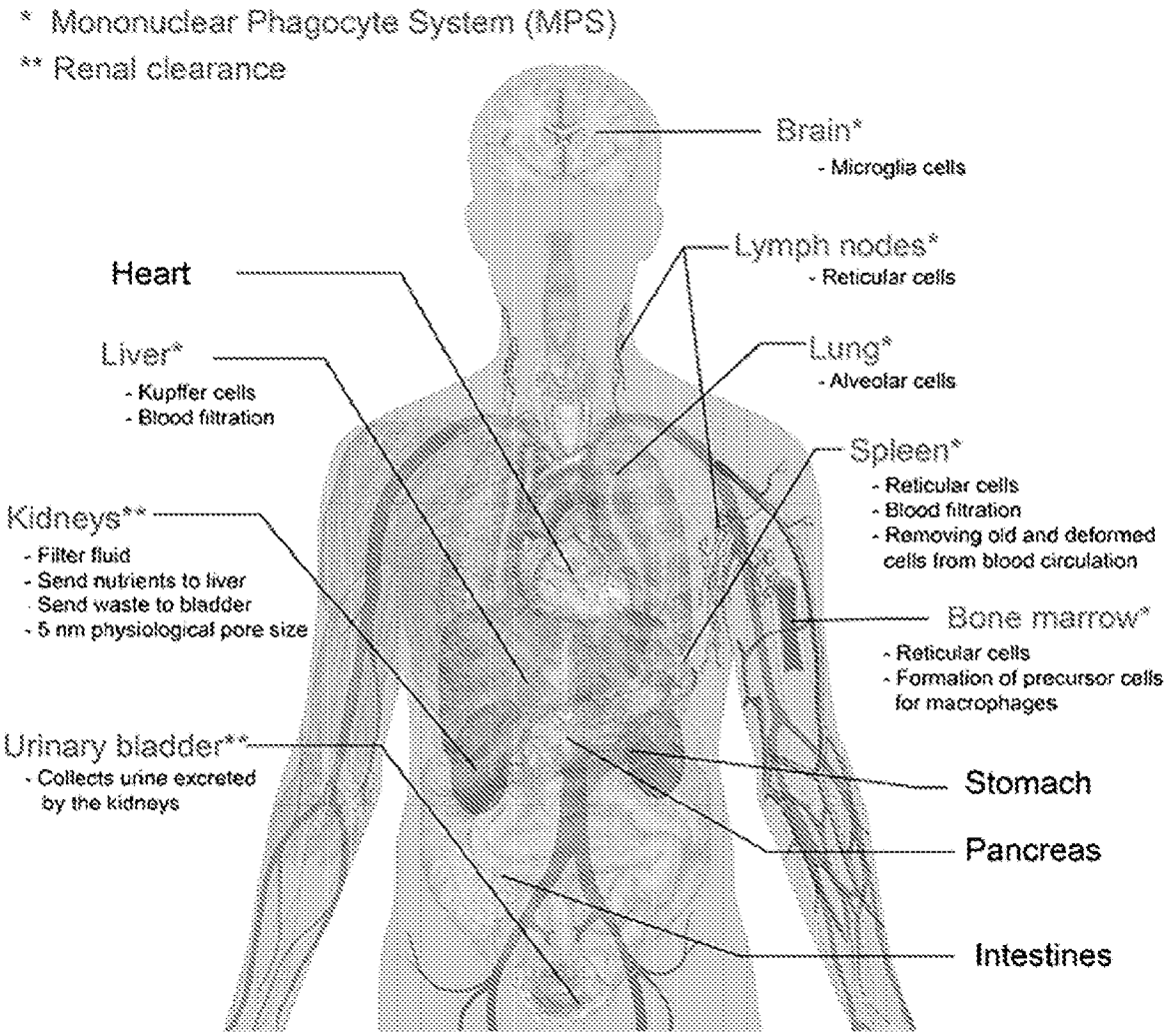

Fig. 4. The mononuclear phagocyte system (MPS). Phagocytes, monocytes, and dendritic cells in the MPS participate in the response to inflammation and infection. Phagocytes are responsible for removing pathogens and foreign bodies such as nanoparticles from circulation. Opsonins that bind to nanoparticles initiate uptake and removal from circulation by phagocytes.

approach to increasing circulation and decreasing elimination half-times is to design the delivery vehicle to appear as "self" as opposed to "non-self" to the immune system [70,71]. All human cells have a unique set of "self" markers, known as the major histocompatibility complex (MHC) to prevent activation of immune cells. These molecules bind to inhibitory receptors, thereby inhibiting cell activation. A marker of "self" that regulates phagocytosis is CD47 which is a ligand for the SIRPα inhibitory receptor [72]. Exploiting markers of "self" may enable the engineering of delivery vehicles to achieve longer circulation times.

4.3. Enhanced permeability and retention (EPR) effect

Tumor vasculature lacks many of the features of normal blood vessels, such as well-defined smooth muscle cells and lymphatic drainage, and is inherently leaky. The preferential accumulation of a molecule or nanoparticle at locations of increased vascular permeability is known as the enhanced permeability and retention (EPR) effect (Fig. 5) [73–77]. A growing solid tumor requires nutrients and metabolites for growth: when the tumor reaches about 1–2 mm^3, the diffusion length and the interstitial pressure increase, restricting the supply of nutrients to the tumor core. The combination of hypoxic environment and inflammatory response leads to the formation of new vessels to supply nutrients to the tumor core, triggered by the expression of angiogenic factors such as vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), and tumor necrosis factor α (TNFα), and downregulation of angiogenic inhibitors, such as thrombospondin-1. This process involves local removal of pericytes, the degradation of basement membrane and extracellular matrix (ECM) by matrix metalloproteinases (MMPs), and the activation of endothelial cells leading to sprouting and the formation of new vessels. Formation of the tumor neovasculature results in a rapid increase in tumor growth rate. In addition, tumor cells release vascular mediators such as bradykinin, prostaglandins, matrix metalloproteinases (MMPs) that increase the paracellular permeability at the junctions between endothelial cells.

As a consequence of the recruitment of blood vessels by the tumor, the neovasculature is not hierarchically organized as in capillary beds but has an irregular architecture and heterogeneous spatial distribution [73,75]. This irregular structure leads to increased resistance to blood flow and poor perfusion. In animal models, the average velocity of red blood cells in tumor neovasculature may be an order of magnitude lower than in normal tissue [78,79]. Degradation of the basement membrane and the lack of smooth muscle cells and pericytes essential for constriction also contribute to leaky vessels. The obstruction and/or collapse of lymphatic vessels at the tumor core reduces drainage and results in accumulation in the local tissue [75,80]. A consequence of poor lymphatic drainage is increased intratumoral pressure.

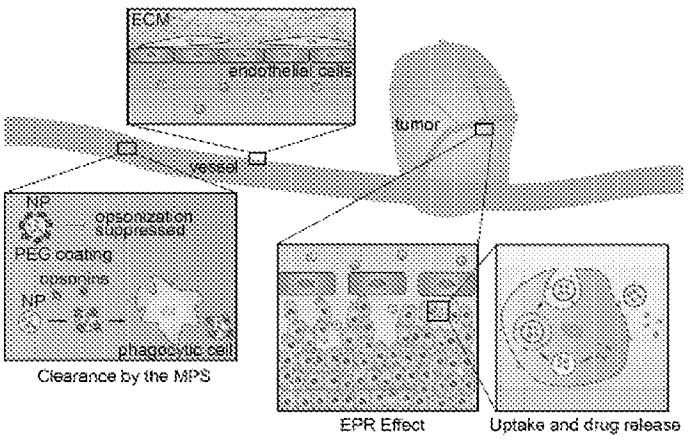

Fig. 5. The Enhanced Permeation and Retention (EPR) effect. The accumulation of a drug delivery platform by the EPR effect requires high sustained plasma concentrations. Minimizing accumulation in peripheral tissue by transendothelial or paracellular transport is important in maintaining high plasma concentrations. Avoiding clearance by the MPS is important in increasing the elimination half-time. Activation of endothelial cells in the tumor vasculature leads to increased permeability compared to normal vasculature. The leakiness of the vasculature is dependent on the tumor type, size, and microenvironment. It is generally assumed that particles less than 200 nm in diameter are able to extravasate to the tumor site. After extravasation to the tumor site, the delivery platform must diffuse to tumor cells and induce cell death. Drug release can occur by various mechanisms and is one of the major challenges in the development of delivery platforms.

The accumulation of nanomedicines and nanoparticles in a tumor by the EPR effect has been demonstrated in animal models [73,81–83]. Evidence for tumor accumulation by the EPR effect in humans is more limited. Fluorescence microscopy of patient biopsies and analysis of tumor interstitial fluid and tumor cells show significantly more doxorubicin in the tumors of patients treated with Doxil compared to free doxorubicin [46,84]. Doxorubicin can undergo passive transcellular transport (lipophilicity $\log P_{oct} = -0.5$, and MW = 543.5) [85] whereas liposomes are limited to paracellular transport at junctions where they are not size excluded. At the same time, after administration of Doxil, more than 98% of the doxorubicin in plasma is in liposomes [22]. Therefore, the increased amount of doxorubicin in patients treated with Doxil supports tumor accumulation of liposomes by the EPR effect.

4.3.1. Heterogeneity

A challenge in exploiting the EPR effect for drug delivery is the inherent physical and biological heterogeneity of the tumor due to the nature of the local microenvironment, tumor type and characteristics, and degree of inflammatory activity [74]. For example, pancreatic tumors are generally very poorly vascularized and hence systemic delivery *via* the EPR effect is thought to be inefficient. As a result of this heterogeneity, the accumulation of a nanoparticle delivery platform at a tumor site is likely to vary considerably from patient to patient. In addition, there is no well-defined size limit for transport across the leaky tumor vasculature. In animal models, the pore size cut-off for extravasation from the tumor vasculature varies from 200 nm to 1.2 μm depending on the tumor type [78,86,87]. In general, a diameter of about 200 nm is usually considered an upper limit for nanoparticle delivery platforms [88].

4.3.2. Enhancing the EPR effect

Various strategies have been explored to modulate the EPR effect to increase drug accumulation in the tumor [73,74]. The EPR effect is involved in inflammation, and hence factors that mediate an inflammatory response can also promote leaky vasculature in tumors [73,74]. For example, bradykinin mediates inflammation and induces extravasation and accumulation of fluids in inflamed tissues. Prostaglandins are upregulated by inflammatory cytokines and prevent platelet aggregation and leukocyte adhesion, thereby enhancing the EPR effect. Nitric oxide (NO) and NO-releasing factors are also important mediators of vascular permeability.

The EPR effect is usually associated with molecules that extravasate from the tumor neovasculature by paracellular transport through the relatively large pores between endothelial cells. Other pathways include passive transport across the cell membranes (usually restricted to small lipophilic molecules ≤ 500 Da), transcytosis *via* an endocytic or receptor mediated pathway, or transport through fenestrations in the endothelial cells.

Accumulation at a tumor site is dependent, in part, on other sinks for the nanoparticle platform. These include renal clearance, clearance by the MPS, and accumulation in peripheral tissue usually through nonspecific binding to the vasculature or highly vascularized tissues. In general, particles that avoid kidney filtration, avoid uptake by the MPS system, and have long circulation half-times are likely to result in significant accumulation in a tumor.

4.3.3. Tumor transport

After extravasation from the neovasculature, nanoparticles enter the interstitial space, usually at the perimeter of the tumor. Therefore, it is the outermost cells of the tumor mass that are first exposed to the

nanoparticle platform. To reach the interior of the tumor, the nanoparticles or free drug must diffuse through the interstitial space between the cancer cells to the tumor core [75,76,87,89]. Depending on the mechanism of drug release from the nanoparticle platform, transport into the tumor may depend on the rate of specific or non-specific binding to the surface of the cancer cells, the rate of uptake by the cells, and the rate of mass transport. The rate of mass transport is dependent on the density of cancer cells in the tumor and hence the effective pore size in the interstitial space. The interstitial space consists of a network of collagen fibers and other proteins and hence transport is also dependent on the size and physicochemical properties of the nanoparticle platform. The average distance from the neovasculature to the tumor core, or the diffusion length, is dependent on the vascular density and tumor size. For poorly vascularized tumors, such as pancreatic neoplasms, the diffusion length is expected to be relatively long and hence the time to reach the tumor core is also expected to be long.

4.4. Drug loading, trafficking and drug release

Drug loading is generally achieved by covalent coupling of the drug to the delivery platform (e.g. ADCs) or by drug encapsulation (e.g. liposomes). Covalent coupling usually requires a cleavable linker that will efficiently release the drug and allow delivery to the appropriate cellular compartment. The various strategies that have been developed for drug encapsulation can be classified by the drug permeability. In the simplest case, a drug can be passively loaded into a porous particle, such as a polymer (e.g. PEI) or inorganic material (e.g. mesoporous silica), where the drug release rate is dependent on the effective pore size. For these systems, the drug continuously diffuses out and hence the platform must be designed to allow sufficient drug concentration after circulation half-times of several days. Drug elution in circulation can be attenuated by engineering the delivery platform to increase the release rate after tumor or cellular uptake. In principle this could be accomplished by externally triggered release, or by exploiting the biochemical conditions in the tumor to trigger release.

The transport of a nanoparticle delivery platform into a cell usually involves binding to the cell surface, translocation across the cell membrane, and intracellular trafficking. As described previously, nanoparticle delivery platforms are usually taken up by an endocytic pathway. Moieties for active targeting, such as transferrin and folic acid, target receptors involved in endocytosis [90,91]. While endocytosis is an efficient method to transport a nanoparticle delivery platform into a cell, release of the drug and escape from endosomes or lysosomes can be challenging. Late stage endosomes and lysosomes have low pH and contain proteolytic enzymes, features that can be exploited for drug cleavage from a delivery vehicle. Nonetheless, design of drug delivery platforms for uptake and release remains largely empirical.

4.5. Toxicity

A key component of the development of a drug therapy is determination of the therapeutic index — the ratio of the dose that results in toxicity in 50% of patients divided by the minimum effective dose in 50% of patients. For targeted nanoparticle drug delivery and/or diagnostic platforms, an additional concern is the potential toxicity of the components of the platform other than the drug [92]. Data from environmental exposure studies can be helpful in guiding design and dosing in initial development. For example, the No Observable Effect Level (NOEL) is the maximum dose with no observable adverse effects, and is usually determined in rats. The Reference Dose (RfD) is an estimate of the daily oral exposure (e.g. mg/kg/day) that is not likely to cause harmful effects during a lifetime. The RfD is usually estimated as NOEL/100. Values for NOEL and RfD for many elements can be found in the literature.

5. Targeting efficiency

The efficacy of a drug or combination drug is often measured in animal models by the time dependence of tumor size or by the fraction of animals that survive after a candidate therapy. These parameters are particularly useful in assessing the potential therapeutic benefit of a new therapy but integrate many factors. An additional parameter that can be useful in assessing the potential efficacy of a targeted drug delivery platform is the targeting efficiency — the fraction of an intravenously administered dose that accumulates in a tumor (%ID). Despite the importance of this parameter, very few measurements are reported in the literature. Unfortunately, results are usually reported as percent of initial administered dose (ID) per gram of tumor (% ID/g), which is only useful if the tumor mass is also reported. The targeting efficiency is expected to be dependent on time post injection and the dose, and hence time-course studies are important to identify and fully characterize the targeting efficiency.

Mouse models are widely used for research studies of disease progression and the development of new therapies [93]. Standard models for targeting experiments include xenografts of human cell lines or explants, orthotopic xenografts, and genetically engineered mouse models [93,94]. While these models are invaluable for preclinical studies of efficacy, pharmacokinetics, and biodistribution, differences in physiology can lead to differences compared to circulation and accumulation of a nanoparticle platform in humans [95]. For example, differences in vascularization can lead to differences in accumulation within tumor. The difference in blood volume between mouse models and humans can also lead to large differences in dilution upon administration.

The targeting efficiency is usually measured using gamma counter, PET, HPLC, or ICP-MS (inductively coupled plasma mass spectroscopy). Methods using a gamma counter or PET require that a suitable radiolabel is conjugated to the drug delivery platform. With a gamma counter, the radioactivity in the resected tumor is measured and compared to the radioactivity of the dose. To determine the targeting efficiency from PET scans, reconstructed 3D regions of interest (ROI) are drawn around the tumor and the activity per unit mass is determined after correcting for decay and tissue density. The targeting efficiency is then determined by comparison to the activity of the initial dose. An alternative to using a radiolabel to measure the targeting efficiency is to use ICP-MS to determine the amount of one or more elemental components in the delivery platform and to compare to the initial dose.

Values of targeting efficiency per gram of tumor (%ID/g) in mouse models for different nanoparticle delivery platforms typically vary from 1% to 10% for both active targeting and passive targeting [96–112]. In some cases, the targeting efficiency per gram of tumor was greater in control experiments without the targeting molecule. However, as described above, it is difficult to make detailed comparisons since the tumor mass may be considerably different.

The lack of consistency in experimental approach makes it very difficult to draw any conclusions from these targeting studies. The difficulties in achieving highly efficient targeting suggest that distribution and elimination elsewhere in the body may be faster than accumulation by the EPR effect. Assessment of the kinetic parameters is difficult, as described previously, due to the heterogeneity of the tumor neovasculature that depends on tumor size and location in the body. In animal models, additional variables include the cell line and/or animal model.

The development of general guidelines for animal studies of nanoparticle delivery platforms would greatly increase the value of research in this field. Key parameters are: amount of initial dose in the tumor, the amount cleared from the body, and the amount in organs (especially liver and spleen) and normal tissue. The time and dose dependencies of these parameters as would be performed in standard pharmacokinetic studies are also important.

6. Design rules for targeted nanoparticle drug delivery platforms

The development of nanoparticle-based delivery platforms to overcome the side effects of systemic delivery is challenging. The inherent difficulty in controlling variables makes it difficult to perform experiments in a way that that allows direct comparison, and as a result, many studies are largely empirical in their design. This is often compounded by the lack of awareness of both the physiological and engineering constraints in the development of nanoparticle delivery platforms. The very few FDA-approved cancer nanomedicines suggest that there is a need for improving our understanding of the physiologically imposed design constraints and the structure–property relations of the engineered nanoparticle platforms.

The general guidelines for nanoparticle delivery platforms are summarized below and in Table 2. These represent general guidelines — for specific applications, some conditions may not be applicable.

- Stability in circulation. The nanoparticle platform should be stable in circulation and should not be degraded or destabilized under flow and at physiological temperature. In addition, the particle should not bind with components of blood that could lead to aggregation, non-specific binding to the endothelium, or uptake by the MPS (see below). To avoid rapid clearance by the kidneys, the delivery platform should be >8 nm.
- Minimize tissue (or peripheral) volume. The distribution of the nanoparticle platform should be limited to the central volume consisting of the blood vessels and tissues highly perfused by blood. Distribution of the nanoparticle platform to normal tissue (peripheral volume) should be minimized. To minimize the peripheral volume, the particle should be designed to minimize specific and non-specific binding to the endothelium that could lead to uptake in normal endothelial cells or trafficking to normal tissue, and to avoid paracellular transport across the endothelium into normal tissue.

To minimize non-specific binding to the endothelium, it is generally thought that particles should have a neutral or slightly negative zeta potential, and should be hydrophilic. Particles should also be designed to avoid binding of components in blood, and in particular, opsonins that promote uptake by phagocytes (see *Evading the MPS*). Binding of components in blood can also lead to aggregation and enhanced non-specific binding to the endothelium.

A potential disadvantage of active targeting platforms is that the arget molecule may be expressed by the normal endothelium. Even if the target molecule is expressed at low levels, the large area of the normal endothelium, compared to the neovasculature or tumor, may result in significant elimination by uptake in normal tissue.

- Evading the MPS. To increase the half-time, the particle should be designed to minimize recognition and clearance by the MPS. Modification of the surface with polyethylene glycol (PEG) is commonly used for this purpose. Minimizing clearance by the MPS will increase the elimination half-time and increase active or passive tumor accumulation by the EPR effect.
- Maximizing tumor accumulation. Tumor accumulation is usually dependent on extravasation of the delivery platform across the tumor vasculature by the EPR effect. To exploit the EPR effect, the nanoparticle diameter should be less than about 200 nm. The rate of accumulation is related to the plasma concentration and the extravasation rate constant. To maintain a high plasma concentration, the nanoparticle platform should be (1) stable in circulation, (2) not accumulate in normal tissue (small peripheral volume), (3) not be cleared by the kidneys, and (4) should evade the MPS. The extravasation rate constant is expected to be highly dependent on the tumor type, location, and the architecture of the tumor neovasculature. One strategy for increasing the extravasation rate constant is to transiently enhance the EPR effect.
- Drug loading. The method of drug loading imposes numerous design constraints on the delivery platform. For example, the continuous drug diffusion from passively loaded delivery platforms makes it more difficult to deliver a therapeutic dose to the tumor and increases toxic side effects in normal tissue. In contrast, drugs that are covalently coupled to the delivery platform must be released in the tumor. A further consideration is the number of drug molecules per delivery platform, which influences the dose. The drug/delivery platform ratio can vary over many orders of magnitude: from 1 to 10^4 or higher. While a high drug/delivery platform ratio can reduce the dose of the delivery platform to achieve a fixed drug dose, this is only effective if a single delivery platform can induce death in many tumor cells.
- Uptake and trafficking. The mechanism of drug action is an important design constraint in defining where the drug must ultimately be delivered. While many anti-cancer drugs target microtubules or DNA in the cell, there are other direct or indirect mechanisms to induce tumor cell death. For many platforms designed for intracellular delivery, uptake occurs by an endocytic pathway. Although endocytic pathways can be very efficient in internalizing these platforms, drug release and endosomal escape are key challenges in achieving high therapeutic efficacy.

7. Future perspectives

The FDA-approved ADCs, liposomal and protein drug delivery platforms overcome a variety of problems associated with very high drug toxicity (Brentuximab vedotin and Trastuzumab emtansine), low

Table 2
Summary of design criteria for nanoparticle drug delivery platforms.

Function	Design requirements	Possible strategies
Circulation	• Stable under flow at 37 °C	• Avoid binding with components of blood • Neutral or slightly negative zeta potential
Distribution	• Minimize tissue (peripheral) volume • Minimize binding to endothelium • Minimize paracellular transport	
Elimination	• Minimize opsonization • Minimize recognition by phagocytic cells of the MPS • Maximize circulation time • Minimize rapid clearance by the kidneys	• Stealth coating • d > 8 nm to avoid rapid clearance in kidneys
Tumor accumulation	• Maximize extravasation across tumor vasculature	• d < 200 nm for transport across leaky vasculature *via* the EPR effect • Maintain high plasma concentration • Enhance EPR effect
Tumor cell uptake	• Maximize binding/uptake by tumor cells	• Active targeting of tumor cells
Drug release	• Drug release/cleavage • Trafficking to cellular compartment	• Active or passive drug release at tumor site • Maximize cell death/ particle; maximize dose/ particle • Maximize endosomal escape for particles taken up by endocytosis

solubility (Abraxane), and side effects associated with high doses of the free drug (Doxil, DaunoXome, Marqibo). The strategies to overcome these problems differ significantly. Doxil liposomes have a polyethylene glycol coating to achieve extended evasion of the MPS, leading to low clearance rates and long elimination half-time. Precipitation of the drug in the liposome increases stability resulting in relatively little free drug in circulation. These features allow accumulation of the liposomes at the tumor site. In contrast DaunoXome, Marqibo, and Abraxane modulate the release of free drug in circulation by phagocytosis (DaunoXome, Marqibo) or dissociation (Abraxane). The antibody–drug conjugates provide active targeting which promotes efficient uptake by tumor cells after extravasation, however, escape from endosomes requires an efficient release strategy.

DaunoXome, Marqibo, and Abraxane are not designed for long circulation half-times, and hence liposomes modified to evade the MPS (e.g. Doxil) and incorporating a targeting moiety represent a logical evolution of current technologies. Nonetheless, active targeting with liposomes, or immunoliposomes, has proven to be extremely difficult. Improved methods for efficient conjugation of targeting moieties that remain active are a critical need in the field.

The FDA-approved ADCs, liposomal and protein drug delivery platforms all rely on the EPR effect for extravasation from the circulation and accumulation at the tumor site. Despite its importance, there remain many gaps in our understanding of the tumor vasculature and methods for maximizing tumor accumulation. Similarly, opportunities for locally enhancing the EPR effect at the site of a tumor or inflammation have not been fully explored.

While the release of the free drug from antibody–drug conjugates is thought to occur in endosomes after endocytosis in tumor cells, the mechanism of drug release by liposomes is not well understood. This is important since the diffusion of large nanoparticles, such as liposomes, in the tumor extracellular space is likely to be much slower than the free drug. Relatively little is known about the release profile for optimum therapeutic efficacy.

In summary, advancing our understanding of the design rules for achieving long circulation times, efficient extravasation and tumor accumulation, and optimum release profiles is likely to be a key to developing the next generation of targeted cancer drug therapies.

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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)

Background: Two combination chemotherapy regimens have emerged as standard of care options for first-line treatment of mPAC: 5-fluorouracil (5-FU)/leucovorin (LV) + irinotecan + oxaliplatin (FOLFIRINOX), and nab-paclitaxel + gemcitabine. nal-IRI (MM-398) is a nanoliposomal formulation of irinotecan. In a randomized Phase 3 study (NAPOLI-1) of patients with mPAC who had been previously treated with gemcitabine-based therapy, nal-IRI + 5-FU/LV demonstrated its safety and significant clinical activity, increasing overall survival (OS) and progression-free survival (PFS) relative to 5-FU/LV. The 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.

Methods: This open-label, Phase 2, comparative study will be conducted in two parts. Part 1 is a safety run-in of a nal-IRI + 5-FU/LV + oxaliplatin regimen. The safety run-in will enroll small cohorts of patients following a traditional 3+3 dose-escalation design to confirm the target dose of oxaliplatin (n = ~6-18). The primary objective of Part 1 is the safety and tolerability of nal-IRI + 5-FU/LV + oxaliplatin. Part 2 is a randomized, efficacy study of a nal-IRI + 5-FU/LV + oxaliplatin regimen (Arm 1), and the nal-IRI + 5-FU/LV combination that previously demonstrated efficacy in the NAPOLI-1 trial (Arm 2) versus a nab-paclitaxel + gemcitabine control arm (Arm 3; total n = ~156-168). The primary objective of Part 2 is to assess the efficacy of nal-IRI–containing regimens in first-line mPAC patients compared with nab-paclitaxel + gemcitabine using the PFS rate at 24 weeks as the primary endpoint. The secondary objective of Part 1 is a PK study; Part 2 secondary endpoints will include OS, PFS, objective response rate (per RECIST, v1.1), decrease in CA19-9 levels, and quality of life assessments.

NCTID: NCT02551991

Poster #TPS-482

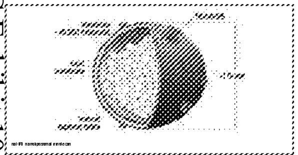
A Randomized, Open-label, Phase 2 Study of Nanoliposomal Irinotecan (nal-IRI)–containing Regimens versus nab-Paclitaxel Plus Gemcitabine in Patients with Previously Untreated, Metastatic Pancreatic Adenocarcinoma (mPAC)

Andrew Dean,[1] Li-Tzong Chen,[2] Ramesh K. Ramanathan,[3] Sarah Blanchette,[4] Bruce Belanger,[4] Deyaa Adib,[4] Eliel Bayever[4]

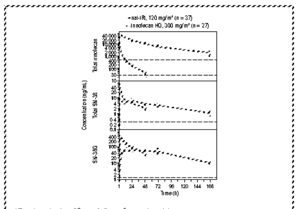
Baxalta

BACKGROUND

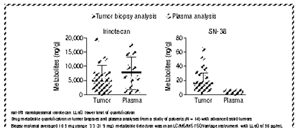
- Two combination chemotherapy regimens have emerged as standard of care for first-line treatment of mPAC:
  - FOLFIRINOX (5-fluorouracil [5-FU] + leucovorin [LV] + irinotecan + oxaliplatin); and
  - nab-paclitaxel + gemcitabine.
- These regimens are associated with median overall survival (OS) of less 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/m²) 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]



nal-IRI, nanoliposomal irinotecan

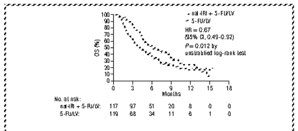


nal-IRI, nanoliposomal irinotecan; AUC, area under the curve; C_{max}, maximum concentration
Comparing nal-IRI with irinotecan HCl: the total irinotecan AUC was 46 times greater and the total irinotecan C_{max} was 13 times greater; the SN-38 AUC was 1.4 times greater and the SN-38 C_{max} was 0.15 times greater. The peak of SN-38 metabolite was lower with nal-IRI versus irinotecan HCl without an increase in SN-38 plasma AUC.



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 (SCIVantage) instrument, with LLoQ of 10 pg/mL for irinotecan and 100 pg/mL for SN-38
Plasma analysis was performed at CPS according to validated procedures with LLoQ of 140 ng/mL for irinotecan and 600 pg/mL for SN-38

- 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.



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

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

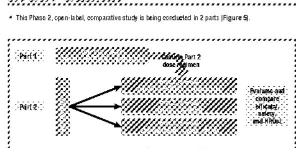
- The primary objective of Part 2 is to compare the PFS rate at 24 weeks in patients in each nal-IRI–containing regimen versus nab-paclitaxel + gemcitabine in first-line mPAC.
- The secondary objectives of Part 2 are to
  - Compare the OS, PFS, and ORR in patients in each nal-IRI–containing regimen relative 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

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

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.

Level	Oxaliplatin Dose (mg/m²)	Dose day	5-FU/LV Dose (mg/m²)	Dose day	nal-IRI 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	60	1, 15
−2B	65	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/m² over 90 minutes [70 mg/m² based on irinotecan free base]) + 5-FU (2,400 mg/m² over 46 hours)/LV (400 mg/m²) on Days 1 and 15 of each cycle; and
    - Arm 3: nab-paclitaxel (125 mg/m² over 35 minutes) + gemcitabine (1,000 mg/m² 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).

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.

Grade 3/4 Toxicity[a]	nal-IRI	5-FU	Oxaliplatin (Arm 1 only)
Neutropenia or febrile neutropenia	1st occurrence: Reduce to 60 mg/m² 2nd occurrence: Reduce to 50 mg/m²	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/m²
Other hematologic toxicity	1st occurrence: Reduce to 60 mg/m² 2nd occurrence: Reduce to 50 mg/m²	1st occurrence: Reduce dose by 25% 2nd occurrence: Reduce dose by another 25%	1st occurrence: Reduce to 60 mg/m² 2nd occurrence: Maintenance of reduced dose at 60 mg/m²
Hand-foot syndrome	1st occurrence: Reduce to 60 mg/m² 2nd occurrence: Reduce to 50 mg/m²	*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/m² *Persistent grade 3, or grade 4:* Discontinue therapy
Other non-hematologic toxicity[b]	1st occurrence: Reduce to 60 mg/m² 2nd occurrence: Reduce to 50 mg/m²	1st occurrence: Reduce dose by 25% 2nd occurrence: Reduce dose by another 25%	1st occurrence: None 2nd occurrence: Reduce to 60 mg/m²

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

Grade 3/4 Toxicity[a]	nab-Paclitaxel	Gemcitabine
Hematologic or non-hematologic toxicity	1st occurrence: Reduce to 100 mg/m² 2nd occurrence: Reduce to 75 mg/m²	1st occurrence: Reduce to 800 mg/m² 2nd occurrence: Reduce to 600 mg/m²
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/mm³; 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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Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial

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Summary

Background Irinotecan is active against colorectal cancer in patients whose disease is refractory to fluorouracil. We investigated the efficacy of these two agents combined for first-line treatment of metastatic colorectal cancer.

Methods 387 patients previously untreated with chemotherapy (other than adjuvant) for advanced colorectal cancer were randomly assigned open-label irinotecan plus fluorouracil and calcium folinate (irinotecan group, n=199) or fluorouracil and calcium folinate alone (no-irinotecan group, n=188). Infusion schedules were once weekly or every 2 weeks, and were chosen by each centre. We assessed response rates and time to progression, and also response duration, survival, and quality of life. Analyses were done on the intention-to-treat population and on evaluable patients.

Findings The response rate was significantly higher in patients in the irinotecan group than in those in the no-irinotecan group (49 *vs* 31%, p<0·001 for evaluable patients, 35 *vs* 22%, p<0·005 by intention to treat). Time to progression was significantly longer in the irinotecan group than in the no-irinotecan group (median 6·7 *vs* 4·4 months, p<0·001), and overall survival was higher (median 17·4 *vs* 14·1 months, p=0·031). Some grade 3 and 4 toxic effects were significantly more frequent in the irinotecan group than in the no-irinotecan group, but effects were predictible, reversible, non-cumulative, and manageable.

Interpretation Irinotecan combined with fluorouracil and calcium folinate was well-tolerated and increased response rate, time to progression, and survival, with a later deterioration in quality of life. This combination should be considered as a reference first-line treatment for metastatic colorectal cancer.

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Introduction

Standard therapy for metastatic colorectal cancer is fluorouracil, commonly modulated by calcium folinate, which typically yields a median survival time of 10–14 months.[1,2] Fluorouracil can be used as first-line or second-line therapy and a different regimen of fluorouracil can be administered as second-line treatment if first-line treatment fails. Survival time in these patients is typically short. Quality of life in patients receiving this treatment is generally poor.[3–10]

Irinotecan inactivates topoisomerase I and thereby inhibits cell division.[11–13] The drug has no cross-resistance with fluorouracil and functions via a novel molecular mechanism.[14] Phase III studies of patients whose disease had not responded to first-line fluorouracil, or patients whose disease had progressed after first-line fluorouracil treatment, showed increased survival times in patients given irinotecan compared with those receiving best supportive care[15] or high-dose fluorouracil and calcium folinate alone by continuous infusion.[16] Data suggest that the development of well-tolerated regimens that combine irinotecan and high-dose fluorouracil and calcium folinate may be beneficial in the first-line treatment of colorectal cancer.

Phase I dose-escalation studies were done to test combined irinotecan with fluorouracil and calcium folinate weekly or every 2 weeks.[17,18] These regimens were selected for first-line treatment of advanced colorectal cancer, based on promising antitumour efficacy and an acceptable safety profile. The choice of regimen was left to investigators, according to local clinical practice, since the two regimens are widely used in Europe. The irinotecan combination and fluorouracil and calcium folinate alone were expected to differ by similar magnitudes in each regimen. Analysis of pooled data for efficacy showed that irinotecan gave a significant survival advantage.

We did a phase III multicentre randomised trial that was designed to assess whether the addition of irinotecan to fluorouracil and calcium folinate would benefit patients previously untreated with chemotherapy (other than adjuvant) for metastatic colorectal cancer.

Methods

Patients

From May, 1997, to February, 1998, we enrolled patients who met the following eligibility criteria: histologically proven adenocarcinoma of the colon or rectum; age 18–75 years; WHO performance status of 2 or less and life expectancy of more than 3 months; haemoglobin 100 g/L or more; absolute neutrophil count $2{\cdot}0\times10^9$/L; platelets 150×10^9/L or more; creatinine 1·25 or less times the upper limit of normal; total bilirubin 1·25 or less times the upper limit of normal; aspartate aminotransferase and alanine aminotransferase 3·0 or less times the upper limit of normal (if liver metastases present, 1·5 or less times for bilirubin and 5·0 or less times for aspartate and alanine

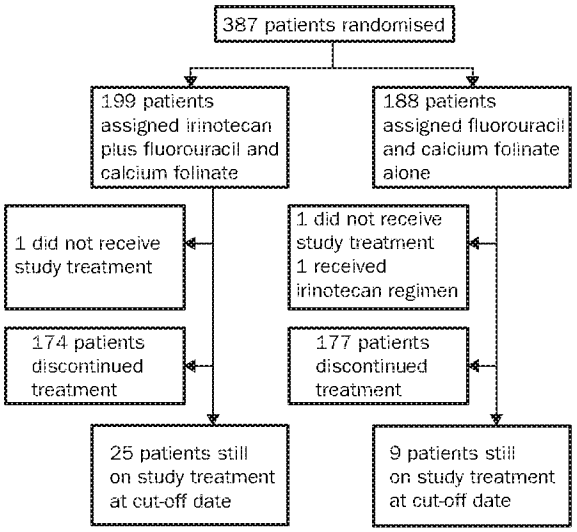

Figure 1: **Trial profile**

aminotransferases); and no previous (other than adjuvant) chemotherapy, finished more than 6 months before randomisation. We obtained written informed consent from each patient before enrolment. Approval was obtained from the ethics committee of each participating centre.

Patients with the following criteria were not eligible: central-nervous-system metastasis, unresolved bowel obstruction or diarrhoea, and known contraindications to fluorouracil (angina pectoris, myocardial infarction in the past 6 months).

Study design

We did the trial in 13 European countries, Israel, and South Africa. We randomly assigned patients irinotecan combined with fluorouracil and calcium folinate (irinotecan group) or fluorouracil and calcium folinate only (no-irinotecan group, figure 1). Randomisation was done centrally in the study sponsor's office by a computer-generated random scheme, and was stratified by centre. Before the start of the study, each investigator chose one of two proposed regimens for fluorouracil and calcium folinate, according to local clinical practice or preference (De Gramont [every 2 weeks] or Arbeitsgemeinschaft Internische Onkologie, cooperative German group for oncology [once weekly]). These regimens were used for combined treatment and fluorouracil and calcium folinate alone.

For the irinotecan group, the regimens were: once weekly, irinotecan 80 mg/m^2 with fluorouracil 2300 mg/m^2 by 24 h infusion, plus calcium folinate 500 mg/m^2 (n=54); or, every 2 weeks, irinotecan 180 mg/m^2 on day 1 with fluorouracil 400 mg/m^2 bolus and 600 mg/m^2 by 22 h infusion, plus calcium folinate 200 mg/m^2 on days 1 and 2 (n=145). For the no-irinotecan group, the regimens were: once-weekly, fluorouracil 2600 mg/m^2 by 24 h infusion plus calcium folinate 500 mg/m^2 (n=43); or every 2 weeks, fluorouracil and calcium folinate at the same doses and administration as in the irinotecan-group 2-weekly regimen (n=143).

Treatment was given until disease progressed, the patient developed unacceptable toxic effects, or consent was withdrawn. Irinotecan was administered according to the guidelines used for irinotecan monotherapy, including recommendations for the use of concurrent antiemetics, atropine, and loperamide. We lowered doses for irinotecan and fluorouracil by 20% if severe toxic effects occurred. At the end of the treatment period, patients were followed up for progression every 3 months. Further cancer treatment was also recorded. During the follow-up period, we traced any continuing adverse effects related to the study treatment until resolution.

Our primary endpoint was response rate. The secondary endpoints for efficacy were time to progression, duration of response, time to treatment failure, and overall survival. Time to progression was the time from randomisation to progression. The duration of response was the time from first infusion to progression in responding patients. The duration of response and stabilisation was the time from first infusion to progression in responding and stable patients. The time to treatment failure was the time from randomisation to treatment discontinuation or progression of disease, whichever came first. Survival lasted from the date of randomisation to the date of death.

Characteristic	Irinotecan group (n=198)	No-irinotecan group (n=187)
Demography		
Male/female	132 (66·7%)/66 (33·3%)	99 (52·9%)/88 (47·1%)
Median (range) age (years)	62·0 (27·0–75·0)	59·0 (24·0–75·0)
WHO performance status		
0	102 (51·5%)	96 (51·3%)
1	83 (41·9%)	77 (41·2%)
2	13 (6·6%)	14 (7·5%)
Weight loss >5%	49 (24·7%)	44 (23·5%)
Number of organs involved		
1–2	169 (85·3)	170 (90·9%)
≥3	29 (14·6%)	17 (9·1%)
Sites of disease		
Liver	152 (76·8%)	149 (79·7%)
Liver alone	89 (44·9%)	93 (49·7%)
Liver plus other sites	63 (31·8%)	56 (29·9%)
Lung	52 (26·3%)	43 (23·0%)
Lymph nodes	28 (14·1%)	24 (12·8%)
Peritoneum/retroperitoneum	20 (10·1%)	22 (11·8%)
Other sites	47 (23·7%)	38 (20·3%)
Time-related variables		
Median (range) time from first metastasis to randomisation (months)	1·4 (0–67·2)	1·6 (0–91·6)
Median (range) time from first diagnosis to first metastasis (months)*	18·9 (3·4–87·2)	21·1 (3·4–102·7)
0–3	110 (55·6%)	121 (64·7%)
3–12	22 (11·1%)	14 (7·5%)
>12	66 (33·3%)	52 (27·8%)
Before treatment		
Synchronous metastases†	110 (55·6%)	121 (64·7%)
Previous adjuvant chemotherapy	51 (25·8%)	44 (23·5%)
At least one tumour-related symptom at baseline	95 (48·0%)	96 (51·3%)
At least one abnormal laboratory value at baseline	177 (89·4%)	157 (84·0%)
CEA >10 ng/mL	144 (72·7%)	128 (68·4%)
Alkaline phosphatase >ULN	89 (44·9%)	75 (40·1%)
Lactase dehydrogenase >ULN	68 (34·3%)	70 (37·4%)

CEA=carcinoembryonic antigen; ULN=upper limit of local laboratory reference range.
*In patients without synchronous metastases. †≤3 months between first diagnosis and first metastasis.

Table 1: **Patients' characteristics at baseline**

Responses were assessed after each treatment cycle, according to WHO criteria. For the weekly regimen, each treatment cycle was 7 weeks and for the 2-weekly regimen, 6 weeks. Tumours were assessed by an external response-review committee, which was masked to treatment group.

We also assessed quality of life. We used the validated QLQ-C30 questionnaire of the European Organization for Research and Treatment of Cancer. The questionnaire includes five scales for functioning, one scale for global health status, and nine symptom scales.[19] Patients completed questionnaires before each cycle (every 6–7 weeks).

Statistical analysis

We needed to include 338 evaluable patients to show a significant difference in response rate between treatment groups, assuming response rates of 35% in the no-irinotecan group and 50% in the irinotecan group, by use of two-tailed χ^2 tests (α=0·05, power 0·80).

Based on the assumption that time to progression would be 6 months in the no-irinotecan group and 9 months in the irinotecan group, we calculated that 286 patients were needed to show a significant difference for this variable. For analysis by two-

	Evaluable population (n=338)		Intention-to-treat population (n=385)	
	Irinotecan group (n=169)	No-irinotecan group (n=169)	Irinotecan group (n=198)	No-irinotecan group (n=187)
Complete reponse	6 (3·6%)	0	6 (3·0%)	0
Partial response	63 (37·3%)	39 (23·1%)	63 (31·8%)	41 (21·9%)
Overall response*	69 (40·8%)	39 (23·1%)	69 (34·8%)	41 (21·9%)
Stable disease	64 (37·9%)	84 (49·7%)	70 (35·4%)	86 (46·0%)
Progressive disease	36 (21·3%)	46 (27·2%)	38 (19·2%)	49 (26·2%)
Not evaluable	0	0	21 (10·6%)	11 (5·9%)

*p<0·001 in evaluable population and p=0·005 in intention-to-treat population.

Table 2: **Response rates**

tailed log-rank test (α=0·05, power of 0·80), with the assumption that accrual and minimum follow-up would last for 6 months and 9 months, respectively, we had to include 143 patients in each treatment group.[20] To accommodate an anticipated 5% loss of patients to follow-up, we planned to include at least 151 patients in each group (total 302).

We analysed the intention-to-treat population and calculated response rates for the intention-to-treat and evaluable populations. χ^2 tests were used to compare categorical variables between treatment groups. For continuous variables, we used Student's *t* tests. We estimated survival curves by the Kaplan-Meier method, and compared the two groups by the log-rank test. We tested for interaction between treatment and regimen by log-rank tests and, additionally, we did a log-rank test stratified by regimen. We constructed a country variable by separating the countries into three classifications, according to the number of patients included. The first group included countries that had recruited more than 30 patients (UK, Spain, France, Czech Republic, Germany, and Austria), the second countries that had recruited 10–30 patients (Belgium, Israel, South Africa, Switzerland, and Italy), and the third countries that had recruited fewer than ten patients (Greece, Portugal, and the Netherlands). We did log-rank tests stratified by this variable.

Multivariate analyses were done on the intention-to-treat population. A logistic-regression model was used to identify the prognostic factors for response. We tested the nine following variables: sex, WHO performance status (<2 and ≥2), weight loss (≤5% and >5%), number of organs involved (<3 and ≥3), primary tumour site (colon or rectum), organ involvement (liver only or other sites), time from first diagnosis to first metastasis (0–3, >3–12, >12 months), previous surgery, and previous adjuvant therapy. For time to progression, Cox's proportional hazards modelling was used, with the following variables: sex, age (<58 and ≥58 years), WHO performance status (<2 and ≥2), weight loss (≤5% and >5%), number of organs involved (<3 and ≥3), primary tumour site (colon or rectum), liver involvement, lymph-node involvement, time from first diagnosis to first metastasis (0–3, >3–12, >12 months), previous surgery, previous adjuvant therapy, time from first diagnosis and first infusion (<9 and ≥9 months). We based the selection of models of prognostic factors on a forward stepwise procedure, with the treatment being forced in the model. To enter and remove terms in the model we used p=0·08 and p=0·10, respectively.

The QLQ-C30 questionnaaire was analysed with the global health status/QoL scale (QL) as the primary endpoint and the other 14 scales as secondary endpoints.

Repeated-measure mixed analysis of variance was used to compare the two treatment groups, treatment and time×treatment being considered as the fixed effects, with time window as a repeated factor and patients as a random factor. To assess the potential bias induced by missing data, we did the same analysis after use of two data-imputation methods, based on the identified reason for data being missing. In each of the methods, dead patients' missing data were imputed as zero scores. Missing data concomitant to grade 3 or 4 adverse events were imputed as the mean of the worst scores from patients with grade 3 or 4 adverse events. In the first imputation method, missing data after progressive disease were imputed as the mean worst scores from progressive patients, and in the second method the last value of the progressive patients was carried forward.

The time to definitive deterioration from baseline by 5%, 10%, 20%, and 30% was analysed by the Kaplan-Meier method. We used log-rank tests to compare the two groups.

Results

Patients and treatment

387 patients were randomly assigned treatment. 385 patients received at least one infusion. Two patients received no study treatment because consent was withdrawn. Of the 385 patients treated in the study, only 97 (25%) received treatment by the weekly regimen (54 in the irinotecan group and 43 in the no-irinotecan group). The other 288 patients received treatment every 2 weeks (145 in the irinotecan group and 143 in the no-irinotecan group).

Baseline demographic and pretreatment characteristics were similar in the two groups (table 1). A higher proportion of women were assigned fluorouracil and calcium folinate alone than the irinotecan regimen, and the rectum was the primary tumour site in a higher proportion of patients in the irinotecan group than in the no-irinotecan group.

Tumour characteristics and history in the two groups were comparable, except for previous surgery, since 89% of patients had had surgery in the irinotecan group and 95% in the no-irinotecan group. The frequency and profile of tumour-related symptoms and laboratory abnormalities were typical of advanced colorectal cancer and similar in the two groups.

The median duration of treatment was longer in the irinotecan than in the no-irinotecan group, irrespective of regimen (24·0 *vs* 21·0 weeks for the weekly regimen, 24·6 *vs* 18·0 for the 2-weekly regimen).

In the irinotecan group, the relative dose intensity was 0·82 for irinotecan and 0·81 for fluorouracil in the weekly regimen, and 0·93 and 0·92, respectively, in the 2-weekly regimen. In the no-irinotecan group, the relative dose intensity was 0·90 in the weekly regimen and 0·96 in the 2-weekly regimen.

39·4% of patients in the irinotecan group and 58·3% in the no-irinotecan group received further chemotherapy; 31·0% of the no-irinotecan group subseqently received irinotecan. Similar proportions of patients received further treatment with oxaliplatin in the two groups (15·7 *vs* 12·8%).

Efficacy

In the evaluable population, the response rate was 49% in the irinotecan group, compared with 31% in the no-irinotecan group (p<0·001). The confirmed responses (after 6–7 weeks) resulted in response rates of 41% (95%

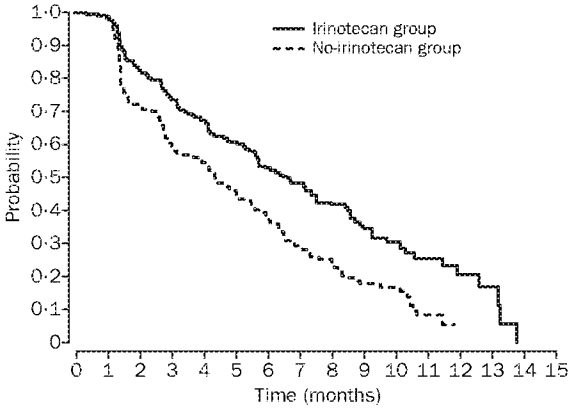

Figure 2: **Time to progression**

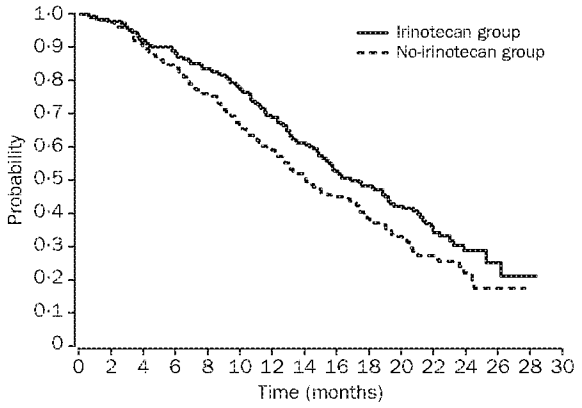

Figure 3: **Survival**

CI 33·3–48·6) and 23% (17·0–30·2), respectively. In the intention-to-treat population, response rate was also significantly higher in the irinotecan group than in the no-irinotecan group (34·8 [28·2–41·9] *vs* 21·9% [16·2–28·5], p=0·005; table 2). The median time to onset of response was 8·9 (range 4·7–25·4) weeks in the irinotecan group and 11·4 (5·3–29·6) weeks in the no-irinotecan group.

The median duration of response was 9·3 (2·8–13·1) months in the irinotecan group and 8·8 (3·7–11·8) months in the no-irinotecan group (p=0·08). Duration of response and stabilisation was longer in the irinotecan group (8·6 [1·6–13·6] *vs* 6·2 [1·1–11·8] months, p<0·001). Time to progression was longer in the irinotecan than in the no-irinotecan group (median 6·7 [0+–13·8+] *vs* 4·4 [0+–11·8+] months, p<0·001; figure 2). The interaction between treatment and regimen was not significant. The log-rank test stratified by regimen and that stratified by country were significant (each p<0·001). Median follow-up was 23·3 (20·0–29·7) months.

Survival in the irinotecan group was significantly longer than in the no-irinotecan group (median 17·4 [0·4–28·4+] *vs* 14·1 [0·5–27·6+] months, p=0·031; figure 3). The probability of survival in the irinotecan group was 82·1% at 9 months and 69·1% at 12 months, and in the no-irinotecan group was 71·6% and 59·1%, respectively. The interaction between treatment and regimen was not significant. This finding supported the hypothesis that the difference in the two regimens would be similar in the two treatment groups and allowed the pooling of data. The log-rank test stratified by regimen was significant (p=0·03), as was that stratified by country (p=0·04).

There was insufficient power to compare the efficacy between groups in the weekly regimen because of the small number of patients who received this regimen. Intention-to-treat analyses showed that for the weekly regimen, the response rates in the irinotecan and no-irinotecan groups did not differ significantly (39·6 [95% CI 26·5–54·0] *vs* 25·0% [13·2–40·3]). Median time to progression was 7·2 (range 0+–13·8) months and 6·5 (0+–12·3+) months. The gradual decrease of the survival curves around 50% did not enable provision of an accurate estimation of the medians.[21] The probability of survival in the irinotecan group was 84·9% at 9 months and 75·5% at 12 months, and in the no-irinotecan group was 77·3% and 62·7%, respectively. In the intention-to-treat analysis of the 2-weekly regimen, the response rate was, for the irinotecan group and the no-irinotecan group, 33·1% (95% CI 25·5–41·4) and 21·0% (14·6–28·6, p=0·021); median time to progression was 6·5 (range 0+–13·2) months and 3·7 (0+–13·1+) months (p=0·001); and median survival was 17·4 (0·4–28·3+) months and 13·0 (0·5–27·6+) months. The log-rank test was significant (p=0·0098). The probability of survival in the irinotecan group was 81·0% at 9 months and 66·7% at 12 months, and in the no-irinotecan group was 69·8% and 54·8%, respectively.

Covariate	Parameter estimate	Wald χ^2	p	Hazard ratio (95% CI)
Treatment group				
No irinotecan				1·00
Irinotecan	0·780	9·558	0·002	2·18 (1·33–3·58)
Weight loss				
>5%				1·00
≤5%	0·804	6·829	0·009	2·23 (1·22–4·08)
Time between first diagnosis and first metastasis (months)				
>12				1·00
3–12	1·001	4·689	0·030	2·72 (1·10–6·73)
0–3	1·063	11·831	0·001	2·90 (1·58–5·31)

Table 3: **Logistic regression of predictive factors for response rate**

Covariate	Parameter estimate	Wald χ^2	p	Hazard ratio (95% CI)
Treatment group				
Irinotecan	..	..	..	1·00
No irinotecan	0·522	15·731	<0·001	1·69 (1·30–2·18)
Number of organs involved				
<3	..	..	..	1·00
≥3	0·443	5·776	0·016	1·56 (1·09–2·23)
Age (years)				
≥58	..	..	..	1·00
<58	0·248	3·643	0·056	1·28 (0·99–1·65)

Table 4: **Cox's model for time to progression**

In the stepwise multivariate logistic regression (table 3), weight loss of 5% or less at baseline and time between first diagnosis and first metastasis were predictive of response. The effect of treatment was significant (p<0·001); the hazard ratio for response was 2·6 times higher in the irinotecan group than in the no-irinotecan group, given the same degree of weight loss at baseline and the same time between first diagnosis and first metastasis.

In Cox's multivariate analysis of time to progression (table 4), age and number of organs involved were significantly predictive. In patients younger than 58 years, the risk of progression increased by about 28%, all other variables being fixed. If three or more organs were involved, the risk of progression was increased by about 56%. The treatment effect was significant (p<0·001). The risk of progression for a patient in the no-irinotecan group

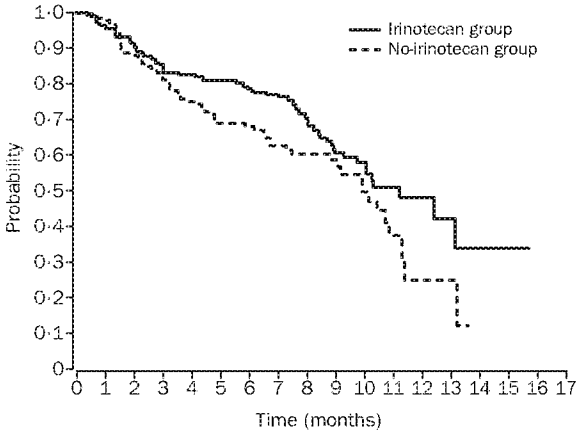

Figure 4: **Time to definitive deterioration in performance status**

Non-haematological toxic effects	Irinotecan group (n=145)		No-irinotecan group (n=143)		p*
	Total	Grade 3 or 4	Total	Grade 3 or 4	
Diarrhoea	48 (88·9%)	24 (44·4%)	28 (65·1%)	11 (25·6%)	0·055
Nausea	39 (72·2%)	4 (7·4%)	25 (58·1%)	2 (4·7%)	0·57
Vomiting	30 (55·6%)	6 (11·1%)	19 (44·2%)	2 (4·7%)	0·25
Asthenia	23 (42·6%)	4 (7·4%)	6 (14·0%)	..	0·068
Alopecia	20 (37·0%)	..	7 (16·3%)	..	..
Anorexia	16 (29·6%)	4 (7·4%)	6 (14·0%)	1 (2·3%)	0·26
Mucositis	14 (25·9%)	..	15 (34·9%)	1 (2·3%)	0·26
Abdominal pain	12 (22·2%)	3 (5·6%)	1 (2·3%)	1 (2·3%)	0·47
Cholinergic syndrome	11 (20·4%)	1 (1·9%)	..	..	0·37
Hand and foot syndrome	9 (16·7%)	..	17 (39·5%)	2 (4·7%)	0·11
Fever in absence of infection without concomitant grade 3–4 neutropenia	6 (11·3%)	..	4 (9·3%)	..	..
Cutaneous signs	4 (7·4%)	..	4 (9·3%)	..	..
Pain	4 (7·4%)	1 (1·9%)	6 (14·0%)	1 (2·3%)	0·87
Weight loss	4 (7·4%)	1 (1·9%)	..	..	0·37
Infection without concomitant grade 3–4 neutropenia	2 (3·7%)	..	2 (4·7%)	..	..
Haematological toxic effects					
Anaemia	51 (94·4%)	3 (5·6%)	41 (97·6%)	..	0·12
Neutropenia	37 (71·2%)	15 (28·8%)	9 (21·4%)	1 (2·4%)	0·001
Leukopenia	40 (74·1%)	11 (20·4%)	16 (38·1%)	1 (2·4%)	0·009
Fever in absence of infection with concomitant grade 3–4 neutropenia	5 (9·3%)	5 (9·3%)	1 (2·3%)	1 (2·4%)	0·16
Infection with concomitant grade 3–4 neutropenia	1 (1·9%)	1 (1·9%)	..	..	0·37

*Based on comparison of frequency of grade 3 or 4 toxic effects.

Table 5: **Patients with any adverse event and with grade 3–4 adverse events related to study treatment (weekly regimen)**

was increased by about 69% compared with that for a patient in the irinotecan group when all other variables were equal.

The median time to treatment failure was 5·3 (0·4–15·7+) months in the irinotecan group and 3·8 (0·4–11·5+) months in the no-irinotecan group (p=0·0014).

The time to definitive deterioration in performance status was significantly longer in the irinotecan group than in the no-irinotecan group (median 11·2 [0·1+–15·7+]) *vs* 9·9 [0+–13·6+] months, p=0·046; figure 4).

Safety

In the irinotecan group, diarrhoea and neutropenia were the most common toxic effects in each regimen, and were significantly more frequent and severe than in the no-irinotecan group.

For toxic effects that occurred at a frequency of 3% or higher, diarrhoea was the most frequent grade 3 or 4 non-haematological toxic effect in each of the treatment groups, irrespective of regimen. Grade 3 and 4 toxic effects occurring at a frequency of 3% or more are shown in tables 5 and 6. With the 2-weekly regimen, diarrhoea was more frequent in the irinotecan group than in the no-irinotecan group, and the difference was close to siginificance in the weekly regimen. This adverse effect occurred more frequently with the weekly than with the corresponding 2-weekly regimen in the two treatment groups. Among patients receiving the weekly regimen, diarrhoea led to hospital admission for 17 (31·5%) in the irinotecan group and five (11·6%) in the no-irinotecan group. For the 2-weekly regimen, 16 (11·0%) patients in the irinotecan group and two (1·4%) in the no-irinotecan group were admitted for diarrhoea. Diarrhoea was the main reason for dose reduction or discontinuation of treatment in the weekly regimen, and neutropenia was the main reason for dose delay in the 2-weekly regimen.

Grade 3 or 4 neutropenia and leukopenia were significantly more frequent in the irinotecan group than in the no-irinotecan group, irrespective of regimen. In the irinotecan group, admission was required for fever in the absence of infection with concomitant grade 3 or 4 neutropenia for five (9·3%) patients on the weekly regimen and one (0·7%) on the 2-weekly regimen; for the no-irinotecan group, admission was required for one (2·3%) patient on the weekly regimen and none on the 2-weekly regimen.

Asthenia was the second most frequent non-haematological toxic effect in the irinotecan group, and grade 3 or 4 asthenia was significantly more frequent in this group than in the no-irinotecan group for the 2-weekly regimen. Grade 3 or 4 infection without grade 3 or 4 neutropenia was significantly more frequent in the irinotecan group than in the no-irinotecan group. In the irinotecan group, about 25% of patients developed cholinergic syndromes that were rarely severe.

Doses were reduced because of toxic effects more frequently for the weekly regimen than for the 2-weekly regimen, and more in the irinotecan than in the no-irinotecan group. Doses were reduced in 29·6% of patients on the weekly regimen and 18·6% on the 2-weekly regimen in the irinotecan and no-irinotecan groups, respectively, and in 20·9% and 4·9% on the 2-weekly regimen.

Most dose reductions occurred during the first two cycles in the weekly regimen. One patient treated with the irinotecan combination on the 2-weekly regimen did not receive appropriate therapy for the management of diarrhoea and died early in the first cycle, probably because of septic shock concomitant with grade 4 neutropenia.

Despite the high frequency of side-effects, in the irinotecan group, the relative dose intensity was preserved compared with the no-irinotecan group.

Quality of life

1161 questionnaires were obtained from the 385 patients in the intention-to-treat population. The rate of return was similar in the two treatment groups—62% in the irinotecan group and 59% in the no-irinotecan group. The two groups did not differ significantly at baseline, except for cognitive function (mean 89·9 [SE 1·1] *vs* 86·1

Non-haematological toxic effects	Irinotecan group (n=54)		No-irinotecan group (n=43)		p*
	Total	Grade 3 or 4	Total	Grade 3 or 4	
Diarrhoea	99 (68·3%)	19 (13·1%)	55 (38·5%)	8 (5·6%)	0·028
Nausea	85 (58·6%)	3 (2·1%)	71 (49·7%)	2 (1·4%)	0·66
Alopecia	82 (56·6%)	..	24 (16·8%)	..	..
Asthenia	65 (44·8%)	9 (6·2%)	50 (35·0%)	1 (0·7%)	0·011
Vomiting	60 (41·4%)	4 (2·8%)	40 (28·0%)	1 (0·7%)	0·18
Mucositis	56 (38·6%)	6 (4·1%)	41 (28·7%)	3 (2·1%)	0·32
Cholinergic syndrome	41 (28·3%)	2 (1·4%)	1 (0·7%)	..	0·16
Anorexia	25 (17·2%)	3 (2·1%)	9 (6·3%)	1 (0·7%)	0·32
Cutaneous signs	16 (11·0%)	1 (0·7%)	24 (16·8%)	..	0·32
Abdominal pain	14 (9·7%)	1 (0·7%)	7 (4·9%)	..	0·32
Hand and foot syndrome	13 (9·0%)	1 (0·7%)	18 (12·6%)	1 (0·7%)	0·99
Pain	12 (8·3%)	..	7 (4·9%)	1 (0·7%)	0·31
Fever in absence of infection without concomitant grade 3–4 neutropenia	9 (6·2%)	..	6 (4·2%)	1 (0·7%)	..
Infection without concomitant grade 3–4 neutropenia	7 (4·8%)	4 (2·8%)	5 (3·5%)	..	0·045
Weight loss	6 (4·1%)	2 (1·4%)	2 (1·4%)	..	0·16
Haematological toxic effects					
Anaemia	140 (97·2%)	3 (2·1%)	130 (90·9%)	3 (2·1%)	0·99
Neutropenia	118 (82·5%)	66 (46·2%)	68 (47·9%)	19 (13·4%)	0·001
Leukopenia	117 (81·3%)	25 (17·4%)	60 (42·0%)	5 (3·5%)	0·001
Fever in absence of infection with concomitant grade 3–4 neutropenia	5 (3·4%)	5 (3·4%)	1 (0·7%)	1 (0·7%)	0·10
Infection with concomitant grade 3–4 neutropenia	3 (2·1%)	3 (2·1%)	..	..	0·08

*Based on comparison of frequency of grade 3 or 4 toxic effects.

Table 6: **Patients with any adverse event and with grade 3–4 adverse events related to study treatment (2-weekly regimen)**

[1·5], p=0·05). QL did not differ significantly between groups. When missing data for death, progressive disease, or grade 3–4 adverse events were taken into account with the two imputation methods, results were biased in favour of the no-irinotecan group. The analysis of variance on QL showed significantly better quality of life in the irinotecan group after the first imputation method was used (p=0·03). The same trend was seen with the second imputation method.

Definitive deterioration in quality of life occurred consistently later in the irinotecan group, for a deterioration from baseline by 5% (p=0·03), 10% (p=0·06), 20% (p=0·04), and 30% (p=0·06).

Discussion

Combination of irinotecan with fluorouracil and calcium folinate significantly increased response rates, time to progression, and survival. The survival advantage was reached, despite a higher proportion of patients receiving further chemotherapy in the no-irinotecan group (58·3 *vs* 39·4%).

This study population was large (385 patients treated), and the patients were representative of candidates for first-line chemotherapy in clinical practice, except that an unexpectedly high proportion of them had synchronous metastases at baseline and, consequently, a relatively low proportion of them had had previous adjuvant chemotherapy.

The higher frequency of adverse events in the irinotecan group than in the no-irinotecan did not affect the median duration of treatment. The rate of grade 3 or 4 diarrhoea was similar to that previously seen with irinotecan administered as a single agent.[15,16] Overall, toxic effects seen with the irinotecan combination treatment were reversible, non-cumulative, and manageable.

No other combination therapy has shown such high antitumour efficacy over high-dose continuous infusion of fluorouracil and calcium folinate to date. In two phase-III studies,[22,23] for oxaliplatin in combination with infusional fluorouracil and calcium folinate, the response rate and progression-free survival were better than with fluorouracil and calcium folinate alone. Those studies did not, however, show survival benefits. Moreover, there was a trend towards shorter survival in the oxaliplatin group in one study.[23] Another phase III study that compared irinotecan combined with bolus fluorouracil and fluorouracil alone[24] consistently provided significantly higher response rates (33 *vs* 18%, p<0·001) and median time to treatment failure (5·0 *vs* 3·8 months, p<0·05) in the irinotecan group.

Moreover, irinotecan combined with fluorouracil and calcium folinate is the only treatment to show a survival advantage in metastatic colorectal cancer over high-dose continuous infusion fluorouracil and calcium folinate alone.

The results we achieved with the irinotecan combination treatment show that the addition of irinotecan to weekly or 2-weekly regimens of fluorouracil and calcium folinate by infusion brings clear clinical benefit and should be considered as a first-line reference treatment in metastatic colorectal cancer as well as for advanced disease.

Contributors

P Rougier and G Gruia designed the trial. P Rougier coordinated the study. J Y Douillard, D Cunningham, A D Roth, M Navarro, R D James, P Karasek, P Jandik, T Iveson, and J Carmichael contributed significantly to accrual. L Awad was responsible for the statistical analysis. M Alaki and G Gruia managed the study, data documentation, and the writing of the study report.

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HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use DOXIL safely and effectively. See full prescribing information for DOXIL.

DOXIL® (doxorubicin hydrochloride liposome injection), for intravenous use
Initial U.S. Approval: 1995

WARNING: CARDIOMYOPATHY and INFUSION-RELATED REACTIONS

See full prescribing information for complete boxed warning.

- **Myocardial damage may lead to congestive heart failure and may occur as the total cumulative dose of doxorubicin HCl approaches 550 mg/m^2. The risk of cardiomyopathy may be increased at lower cumulative doses with mediastinal irradiation (5.1).**
- **Acute infusion-related reactions occurred in 11% of patients with solid tumors. Serious, life-threatening, and fatal infusion reactions have been reported. Medications/emergency equipment to treat such reactions should be available for immediate use (5.2).**

RECENT MAJOR CHANGES

Boxed Warning	01/2015
Dosage and Administration (2)	01/2015
Contraindications (4)	01/2015
Warnings and Precautions (5)	01/2015

INDICATIONS AND USAGE

DOXIL is an anthracycline topoisomerase II inhibitor indicated for:

- **Ovarian cancer (1.1)**

After failure of platinum-based chemotherapy.

- **AIDS-related Kaposi's Sarcoma (1.2)**

After failure of prior systemic chemotherapy or intolerance to such therapy.

- **Multiple Myeloma (1.3)**

In combination with bortezomib in patients who have not previously received bortezomib and have received at least one prior therapy.

DOSAGE AND ADMINISTRATION

Administer DOXIL at an initial rate of 1 mg/min to minimize the risk of infusion reactions. If no infusion related reactions occur, increase rate of infusion to complete administration over 1 hour. Do not administer as bolus injection or undiluted solution (2).

- **Ovarian cancer:** 50 mg/m^2 IV every 4 weeks (2.2)
- **AIDS-related Kaposi's Sarcoma:** 20 mg/m^2 IV every 3 weeks (2.3)
- **Multiple Myeloma:** 30 mg/m^2 IV on day 4 following bortezomib (2.4)

DOSAGE FORMS AND STRENGTHS

Doxorubicin hydrochloride (HCl) liposomal injection: Single use vials. 20 mg/10 mL and 50 mg/25 mL (3)

CONTRAINDICATIONS

- Hypersensitivity reactions to doxorubicin HCl or the components of DOXIL (4, 5.2)

WARNINGS AND PRECAUTIONS

- Hand-Foot Syndrome may occur. Dose modification or discontinuation may be required (5.3)
- Embryofetal Toxicity: Can cause fetal harm. Advise of potential risk to a fetus. Use effective contraception (5.5, 8.1, 8.3)

ADVERSE REACTIONS

Most common adverse reactions (>20%) are asthenia, fatigue, fever, anorexia, nausea, vomiting, stomatitis, diarrhea, constipation, hand-foot syndrome, rash, neutropenia, thrombocytopenia, and anemia (6).

To report SUSPECTED ADVERSE REACTIONS contact Janssen Products, LP at 1-800-JANSSEN (1-800-526-7736) or FDA at 1-800-FDA-1088 or *www.fda.gov/medwatch*.

USE IN SPECIFIC POPULATIONS

- Lactation: Discontinue breastfeeding (8.2).

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 04/2015

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FULL PRESCRIBING INFORMATION

WARNING: CARDIOMYOPATHY and INFUSION-RELATED REACTIONS

- **DOXIL (doxorubicin HCl liposome injection) can cause myocardial damage, including congestive heart failure, as the total cumulative dose of doxorubicin HCl approaches 550 mg/m^2. In a clinical study of 250 patients with advanced cancer who were treated with DOXIL, the risk of cardiotoxicity was 11% when the cumulative anthracycline dose was between 450-550 mg/m^2. Prior use of other anthracyclines or anthracenediones should be included in calculations of total cumulative dosage. The risk of cardiomyopathy may be increased at lower cumulative doses in patients with prior mediastinal irradiation *[see Warnings and Precautions (5.1)]*.**
- **Acute infusion-related reactions consisting of, but not limited to, flushing, shortness of breath, facial swelling, headache, chills, back pain, tightness in the chest or throat, and/or hypotension occurred in 11% of patients with solid tumors treated with DOXIL. Serious, life-threatening and fatal infusion reactions have been reported *[see Dosage and Administration (2.6) and Warnings and Precautions (5.2)]*.**

1 INDICATIONS AND USAGE

1.1 Ovarian Cancer

DOXIL is indicated for the treatment of patients with ovarian cancer whose disease has progressed or recurred after platinum-based chemotherapy.

1.2 AIDS-Related Kaposi's Sarcoma

DOXIL is indicated for the treatment of AIDS-related Kaposi's sarcoma in patients after failure of prior systemic chemotherapy or intolerance to such therapy.

1.3 Multiple Myeloma

DOXIL, in combination with bortezomib, is indicated for the treatment of patients with multiple myeloma who have not previously received bortezomib and have received at least one prior therapy.

2 DOSAGE AND ADMINISTRATION

2.1 Important Use Information

Do not substitute DOXIL for doxorubicin HCl injection.

Do not administer as an undiluted suspension or as an intravenous bolus *[see Warnings and Precautions (5.2)]*.

2.2 Ovarian Cancer

The recommended dose of DOXIL is 50 mg/m^2 intravenously over 60 minutes every 28 days until disease progression or unacceptable toxicity.

2.3 AIDS-Related Kaposi's Sarcoma

The recommended dose of DOXIL is 20 mg/m^2 intravenously over 60 minutes every 21 days until disease progression or unacceptable toxicity.

2.4 Multiple Myeloma

The recommended dose of DOXIL is 30 mg/m^2 intravenously over 60 minutes on day 4 of each 21-day cycle for eight cycles or until disease progression or unacceptable toxicity. Administer DOXIL after bortezomib on day 4 of each cycle *[see Clinical Studies (14.3)]*.

2.5 Dose Modifications for Adverse Reactions

Do not increase DOXIL after a dose reduction for toxicity.

Table 1: Recommended Dose Modifications for Hand-Foot Syndrome, Stomatitis, or Hematologic Adverse Reactions

Toxicity	Dose Adjustment
Hand-Foot Syndrome (HFS)	
Grade 1: Mild erythema, swelling, or desquamation not interfering with daily activities	• If no previous Grade 3 or 4 HFS: no dose adjustment. • If previous Grade 3 or 4 HFS: delay dose up to 2 weeks, then decrease dose by 25%.
Grade 2: Erythema, desquamation, or swelling interfering with, but not precluding normal physical activities; small blisters or ulcerations less than 2 cm in diameter	• **Delay dosing up to 2 weeks or until resolved to Grade 0-1.** • Discontinue DOXIL if no resolution after 2 weeks. • If resolved to Grade 0-1 within 2 weeks: o And no previous Grade 3 or 4 HFS: continue treatment at previous dose. o And previous Grade 3 or 4 toxicity: decrease dose by 25%.
Grade 3: Blistering, ulceration, or swelling interfering with walking or normal daily activities; cannot wear regular clothing	• **Delay dosing up to 2 weeks or until resolved to Grade 0-1,** then decrease dose by 25%. • Discontinue DOXIL if no resolution after 2 weeks.
Grade 4: Diffuse or local process causing infectious complications, or a bed ridden state or hospitalization	• **Delay dosing up to 2 weeks or until resolved to Grade 0-1,** then decrease dose by 25%. • Discontinue DOXIL if no resolution after 2 weeks.
Stomatitis	
Grade 1: Painless ulcers, erythema, or mild soreness	• If no previous Grade 3 or 4 toxicity: no dose adjustment. • If previous Grade 3 or 4 toxicity: delay up to 2 weeks then decrease dose by 25%.
Grade 2: Painful erythema, edema, or ulcers, but can eat	• **Delay dosing up to 2 weeks or until resolved to Grade 0-1.** • Discontinue DOXIL if there is no resolution after 2 weeks. • If resolved to Grade 0-1 within 2 weeks: o And no previous Grade 3 or 4 stomatitis: resume treatment at previous dose. o And previous Grade 3 or 4 toxicity: decrease dose by 25%.
Grade 3: Painful erythema, edema, or ulcers, and cannot eat	• **Delay dosing up to 2 weeks or until resolved to Grade 0-1.** Decrease dose by 25% and return to original dose interval. • If after 2 weeks there is no resolution, discontinue DOXIL.
Grade 4: Requires parenteral or enteral support	• **Delay dosing up to 2 weeks or until resolved to Grade 0-1.** Decrease dose by 25% and return to original dose interval. • If after 2 weeks there is no resolution, discontinue DOXIL.
Neutropenia or Thrombocytopenia	
Grade 1	No dose reduction
Grade 2	Delay until ANC ≥ 1,500 and platelets ≥ 75,000; resume treatment at previous dose
Grade 3	Delay until ANC ≥ 1,500 and platelets ≥ 75,000; resume treatment at previous dose
Grade 4	Delay until ANC ≥ 1,500 and platelets ≥ 75,000; resume at 25% dose reduction or continue previous dose with prophylactic granulocyte growth factor

Table 2: Recommended Dose Modifications of DOXIL for Toxicity When Administered in Combination With Bortezomib

Toxicity	DOXIL
Fever ≥38°C and ANC <1,000/mm^3	• Withhold dose for this cycle if before Day 4; • Decrease dose by 25%, if after Day 4 of previous cycle.
On any day of drug administration after Day 1 of each cycle: • Platelet count <25,000/mm^3 • Hemoglobin <8 g/dL • ANC <500/mm^3	• Withhold dose for this cycle if before Day 4; • Decrease dose by 25%, if after Day 4 of previous cycle AND if bortezomib is reduced for hematologic toxicity.
Grade 3 or 4 non-hematologic drug related toxicity	Do not dose until recovered to Grade <2, then reduce dose by 25%.

For neuropathic pain or peripheral neuropathy, no dosage adjustments are required for DOXIL. Refer to bortezomib manufacturer's prescribing information.

2.6 Preparation and Administration

Preparation

Dilute DOXIL doses up to 90 mg in 250 mL of 5% Dextrose Injection, USP prior to administration. Dilute doses exceeding 90 mg in 500 mL of 5% Dextrose Injection, USP prior to administration. Refrigerate diluted DOXIL at 2°C to 8°C (36°F to 46°F) and administer within 24 hours.

Administration

Inspect parenteral drug products visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use if a precipitate or foreign matter is present.

Do not use with in-line filters.

Administer the first dose of DOXIL at an initial rate of 1 mg/min. If no infusion-related adverse reactions are observed, increase the infusion rate to complete the administration of the drug over one hour *[see Warnings and Precautions (5.2)]*. Do not rapidly flush the infusion line.

Do not mix DOXIL with other drugs.

Management of Suspected Extravasation

Discontinue DOXIL for burning or stinging sensation or other evidence indicating perivenous infiltration or extravasation. Manage confirmed or suspected extravasation as follows:

- Do not remove the needle until attempts are made to aspirate extravasated fluid

- Do not flush the line
- Avoid applying pressure to the site
- Apply ice to the site intermittently for 15 min 4 times a day for 3 days
- If the extravasation is in an extremity, elevate the extremity

2.7 Procedure for Proper Handling and Disposal

DOXIL is a cytotoxic drug. Follow applicable special handling and disposal procedures.[1] If DOXIL comes into contact with skin or mucosa, immediately wash thoroughly with soap and water.

3 DOSAGE FORMS AND STRENGTHS

DOXIL: doxorubicin HCl liposomal injection: single use vials contain 20 mg/10 mL and 50 mg/25 mL doxorubicin HCl as a translucent, red liposomal dispersion.

4 CONTRAINDICATIONS

DOXIL is contraindicated in patients who have a history of severe hypersensitivity reactions, including anaphylaxis, to doxorubicin HCl *[see Warnings and Precautions (5.2)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Cardiomyopathy

Doxorubicin HCl can result in myocardial damage, including acute left ventricular failure. The risk of cardiomyopathy with doxorubicin HCl is generally proportional to the cumulative exposure. The relationship between cumulative DOXIL dose and the risk of cardiac toxicity has not been determined.

In a clinical study in 250 patients with advanced cancer who were treated with DOXIL, the risk of cardiotoxicity was 11% when the cumulative anthracycline dose was between 450-550 mg/m^2. Cardiotoxicity was defined as >20% decrease in resting left ventricular ejection fraction (LVEF) from baseline where LVEF remained in the normal range or a >10% decrease in LVEF from baseline where LVEF was less than the institutional lower limit of normal. Two percent of patients developed signs and symptoms of congestive heart failure without documented evidence of cardiotoxicity.

Assess left ventricular cardiac function (e.g. MUGA or echocardiogram) prior to initiation of DOXIL, during treatment to detect acute changes, and after treatment to detect delayed cardiotoxicity. Administer DOXIL to patients with a history of cardiovascular disease only when the potential benefit of treatment outweighs the risk.

5.2 Infusion-Related Reactions

Serious and sometimes life-threatening infusion-related reactions characterized by one or more of the following symptoms can occur with DOXIL: flushing, shortness of breath, facial swelling, headache, chills, chest pain, back pain, tightness in the chest and throat, fever, tachycardia, pruritus, rash, cyanosis, syncope, bronchospasm, asthma, apnea, and hypotension. The majority of infusion-related events occurred during the first infusion. Of 239 patients with ovarian cancer treated with DOXIL in Trial 4, 7% of patients experienced acute infusion-related reactions resulting in dose interruption. All occurred during cycle 1 and none during subsequent cycles. Across multiple studies of DOXIL monotherapy including this and other studies enrolling 760 patients with various solid tumors, 11% of patients had infusion-related reactions.

Ensure that medications to treat infusion-related reactions and cardiopulmonary resuscitative equipment are available for immediate use prior to initiation of DOXIL. Initiate DOXIL infusions at a rate of 1 mg/min and increase rate as tolerated *[see Dosage and Administration (2.6)]*. In the event of an infusion-related reaction, temporarily stop the drug until resolution then resume at a reduced infusion rate. Discontinue DOXIL infusion for serious or life-threatening infusion-related reactions.

5.3 Hand-Foot Syndrome (HFS)

In Trial 4, the incidence of HFS was 51% of patients in the DOXIL arm and 0.9% of patients in the topotecan arm, including 24% Grade 3 or 4 cases of HFS in DOXIL-treated patients and no Grade 3 or 4 cases in topotecan-treated patients. HFS or other skin toxicity required discontinuation of DOXIL in 4.2% of patients.

HFS was generally observed after 2 or 3 cycles of treatment but may occur earlier. Delay DOXIL for the first episode of Grade 2 or greater HFS *[see Dosage and Administration (2.5)]*. Discontinue DOXIL if HFS is severe and debilitating.

5.4 Secondary Oral Neoplasms

Secondary oral cancers, primarily squamous cell carcinoma, have been reported from post-marketing experience in patients with long-term (more than one year) exposure to DOXIL. These malignancies were diagnosed both during treatment with DOXIL and up to 6 years after the last dose. Examine patients at regular intervals for the presence of oral ulceration or with any oral discomfort that may be indicative of secondary oral cancer.

The altered pharmacokinetics and preferential tissue distribution of liposomal doxorubicin that contributes to enhanced skin toxicity and mucositis compared to free doxorubicin may play a role in the development of oral secondary malignancies with long-term use.

5.5 Embryofetal Toxicity

Based on animal data, DOXIL can cause fetal harm when administered to a pregnant woman. At doses approximately 0.12 times the recommended clinical dose, DOXIL was embryotoxic and abortifacient in rabbits. Advise pregnant women of the potential risk to a fetus. Advise females and males of reproductive potential to use effective contraception during and for 6 months after treatment with DOXIL *[see Use in Specific Populations (8.1) and (8.3)]*.

6 ADVERSE REACTIONS

The following adverse reactions are discussed in more detail in other sections of the labeling.

- Cardiomyopathy *[see Warnings and Precautions (5.1)]*
- Infusion-Related Reactions *[see Warnings and Precautions (5.2)]*
- Hand-Foot Syndrome *[see Warnings and Precautions (5.3)]*
- Secondary Oral Neoplasms *[see Warnings and Precautions (5.4)]*

The most common adverse reactions (>20%) observed with DOXIL are asthenia, fatigue, fever, nausea, stomatitis, vomiting, diarrhea, constipation, anorexia, hand-foot syndrome, rash and neutropenia, thrombocytopenia and anemia.

6.1 Adverse Reactions in Clinical Trials

Because clinical trials are conducted under widely varying conditions, the adverse reaction rates observed cannot be directly compared to rates on other clinical trials and may not reflect the rates observed in clinical practice.

The safety data reflect exposure to DOXIL in 1310 patients including: 239 patients with ovarian cancer, 753 patients with AIDS-related Kaposi's sarcoma, and 318 patients with multiple myeloma.

The following tables present adverse reactions from clinical trials of single-agent DOXIL in ovarian cancer and AIDS-Related Kaposi's sarcoma.

Patients With Ovarian Cancer

The safety data described below are from Trial 4, which included 239 patients with ovarian cancer treated with DOXIL 50 mg/m^2 once every 4 weeks for a minimum of four courses in a randomized, multicenter, open-label study. In this trial, patients received DOXIL for a median number of 3.2 months (range 1 day to 25.8 months). The median age of the patients is 60 years (range 27 to 87), with 91% Caucasian, 6% Black, and 3% Hispanic or Other.

Table 3 presents the hematologic adverse reactions from Trial 4.

Table 3: Hematologic Adverse Reactions in Trial 4

	DOXIL Patients (n=239)	Topotecan Patients (n=235)
Neutropenia		
500 - <1000/mm^3	8%	14%
<500/mm^3	4.2%	62%
Anemia		
6.5 - <8 g/dL	5%	25%
< 6.5 g/dL	0.4%	4.3%
Thrombocytopenia		
10,000 - <50,000/mm^3	1.3%	17%
<10,000/mm^3	0.0%	17%

Table 4 presents the non-hematologic adverse reactions from Trial 4.

Table 4: Non-Hematologic Adverse Reactions in Trial 4

Non-Hematologic Adverse Reaction 10% or Greater	DOXIL (%) treated (n=239)		Topotecan (%) treated (n=235)	
	All grades	Grades 3-4	All grades	Grades 3-4
Body as a Whole				
Asthenia	40	7	52	8
Fever	21	0.8	31	6
Mucous Membrane Disorder	14	3.8	3.4	0
Back Pain	12	1.7	10	0.9
Infection	12	2.1	6	0.9
Headache	11	0.8	15	0
Digestive				
Nausea	46	5	63	8
Stomatitis	41	8	15	0.4
Vomiting	33	8	44	10
Diarrhea	21	2.5	35	4.2
Anorexia	20	2.5	22	1.3
Dyspepsia	12	0.8	14	0
Nervous				
Dizziness	4.2	0	10	0
Respiratory				
Pharyngitis	16	0	18	0.4
Dyspnea	15	4.1	23	4.3
Cough increased	10	0	12	0
Skin and Appendages				
Hand-foot syndrome	51	24	0.9	0
Rash	29	4.2	12	0.4
Alopecia	19	N/A	52	N/A

The following additional adverse reactions were observed in patients with ovarian cancer with doses administered every four weeks (Trial 4).

Incidence 1% to 10%

Cardiovascular: vasodilation, tachycardia, deep vein thrombosis, hypotension, cardiac arrest.

Digestive: oral moniliasis, mouth ulceration, esophagitis, dysphagia, rectal bleeding, ileus.

Hematologic and Lymphatic: ecchymosis.

Metabolic and Nutritional: dehydration, weight loss, hyperbilirubinemia, hypokalemia, hypercalcemia, hyponatremia.

Nervous: somnolence, dizziness, depression.

Respiratory: rhinitis, pneumonia, sinusitis, epistaxis.

Skin and Appendages: pruritus, skin discoloration, vesiculobullous rash, maculopapular rash, exfoliative dermatitis, herpes zoster, dry skin, herpes simplex, fungal dermatitis, furunculosis, acne.

Special Senses: conjunctivitis, taste perversion, dry eyes.

Urinary: urinary tract infection, hematuria, vaginal moniliasis.

Patients With AIDS-Related Kaposi's Sarcoma

The safety data described is based on the experience reported in 753 patients with AIDS-related Kaposi's sarcoma (KS) enrolled in four open-label, uncontrolled trials of DOXIL administered at doses ranging from 10 to 40 mg/m^2 every 2 to 3 weeks. Demographics of the population were: median age 38.7 years (range 24-70); 99% male; 88% Caucasian, 6% Hispanic, 4% Black, and 2% Asian/other/unknown. The majority of patients were treated with 20 mg/m^2 of DOXIL every 2 to 3 weeks with a median exposure of 4.2 months (range 1 day to 26.6 months). The median cumulative dose was 120 mg/m^2 (range 3.3 to 798.6 mg/m^2); 3% received cumulative doses of greater than 450 mg/m^2.

Disease characteristics were: 61% poor risk for KS tumor burden, 91% poor risk for immune system, and 47% poor risk for systemic illness; 36% were poor risk for all three categories; median CD4 count 21 cells/mm^3 (51% less than 50 cells/mm^3); mean absolute neutrophil count at study entry approximately 3,000 cells/mm^3.

Of the 693 patients with concomitant medication information, 59% were on one or more antiretroviral medications [35% zidovudine (AZT), 21% didanosine (ddI), 16% zalcitabine (ddC), and 10% stavudine (D4T)]; 85% received PCP prophylaxis (54% sulfamethoxazole/trimethoprim); 85% received antifungal medications (76% fluconazole),

72% received antivirals (56% acyclovir, 29% ganciclovir, and 16% foscarnet) and 48% patients received colony-stimulating factors (sargramostim/filgrastim) during their course of treatment.

Adverse reactions led to discontinuation of treatment in 5% of patients with AIDS-related Kaposi's sarcoma and included myelosuppression, cardiac adverse reactions, infusion-related reactions, toxoplasmosis, HFS, pneumonia, cough/dyspnea, fatigue, optic neuritis, progression of a non-KS tumor, allergy to penicillin, and unspecified reasons. Tables 5 and 6 summarize adverse reactions reported in patients treated with DOXIL for AIDS-related Kaposi's sarcoma in a pooled analysis of the four trials.

Table 5: Hematologic Adverse Reactions Reported in Patients With AIDS-Related Kaposi's Sarcoma

	Patients With Refractory or Intolerant AIDS-Related Kaposi's Sarcoma (n=74*)	Total Patients With AIDS-Related Kaposi's Sarcoma (n=720**)
Neutropenia		
$< 1000/mm^3$	46%	49%
$< 500/mm^3$	11%	13%
Anemia		
< 10 g/dL	58%	55%
< 8 g/dL	16%	18%
Thrombocytopenia		
$< 150,000/mm^3$	61%	61%
$< 25,000/mm^3$	14%	4.2%

*This includes a subset of subjects who were retrospectively identified as having disease progression on prior systemic combination chemotherapy (at least 2 cycles of a regimen containing at least 2 of 3 treatments: bleomycin, vincristine or vinblastine, or doxorubicin) or as being intolerant to such therapy.
**This includes only subjects with AIDS-KS who had available data from the 4 pooled trials.

Table 6: Non-Hematologic Adverse Reactions Reported in ≥ 5% of Patients With AIDS-Related Kaposi's Sarcoma

Adverse Reactions	Patients With Refractory or Intolerant AIDS-Related Kaposi's Sarcoma (n=77*)	Total Patients With AIDS-Related Kaposi's Sarcoma (n=705**)
Nausea	18%	17%
Asthenia	7%	10%
Fever	8%	9%
Alopecia	9%	9%
Alkaline Phosphatase Increase	1.3%	8%
Vomiting	8%	8%
Diarrhea	5%	8%
Stomatitis	5%	7%
Oral Moniliasis	1.3%	6%

*This includes a subset of subjects who were retrospectively identified as having disease progression on prior systemic combination chemotherapy (at least 2 cycles of a regimen containing at least 2 of 3 treatments: bleomycin, vincristine or vinblastine, or doxorubicin) or as being intolerant to such therapy.
**This includes only subjects with AIDS-KS who had available adverse event data from the 4 pooled trials.

The following additional adverse reactions were observed in 705 patients with AIDS-related Kaposi's sarcoma.

Incidence 1% to 5%

Body as a Whole: headache, back pain, infection, allergic reaction, chills.

Cardiovascular: chest pain, hypotension, tachycardia.

Cutaneous: herpes simplex, rash, itching.

Digestive: mouth ulceration, anorexia, dysphagia.

Metabolic and Nutritional: SGPT increase, weight loss, hyperbilirubinemia.

Other: dyspnea, pneumonia, dizziness, somnolence.

Incidence Less Than 1%

Body As A Whole: sepsis, moniliasis, cryptococcosis.

Cardiovascular: thrombophlebitis, cardiomyopathy, palpitation, bundle branch block, congestive heart failure, heart arrest, thrombosis, ventricular arrhythmia.

Digestive: hepatitis.

Metabolic and Nutritional Disorders: dehydration.

Respiratory: cough increase, pharyngitis.

Skin and Appendages: maculopapular rash, herpes zoster.

Special Senses: taste perversion, conjunctivitis.

Patients With Multiple Myeloma

The safety data described are from 318 patients treated with DOXIL (30 mg/m^2) administered on day 4 following bortezomib (1.3 mg/m^2 i.v. bolus on days 1, 4, 8 and 11) every 3 weeks, in a randomized, open-label, multicenter study (Trial 6). In this trial, patients in the DOXIL + bortezomib combination group were treated for a median number of 4.5 months (range 21 days to 13.5 months). The population was 28 to 85 years of age (median age 61), 58% male, 90% Caucasian, 6% Black, and 4% Asian and Other. Table 7 lists adverse reactions reported in 10% or more of patients treated with DOXIL in combination with bortezomib for multiple myeloma.

Table 7: Frequency of Treatment-Emergent Adverse Reactions Reported in ≥10% Patients Treated for Multiple Myeloma With DOXIL in Combination With Bortezomib

Adverse Reaction	DOXIL + bortezomib (n=318)		Bortezomib (n=318)	
	Any (%)	Grade 3-4	Any (%)	Grade 3-4
Blood and lymphatic system disorders				
Neutropenia	36	32	22	16
Thrombocytopenia	33	24	28	17
Anemia	25	9	21	9
General disorders and administration site conditions				
Fatigue	36	7	28	3
Pyrexia	31	1	22	1
Asthenia	22	6	18	4
Gastrointestinal disorders				
Nausea	48	3	40	1
Diarrhea	46	7	39	5
Vomiting	32	4	22	1
Constipation	31	1	31	1
Mucositis/Stomatitis	20	2	5	<1
Abdominal pain	11	1	8	1
Infections and infestations				
Herpes zoster	11	2	9	2
Herpes simplex	10	0	6	1
Investigations				
Weight decreased	12	0	4	0
Metabolism and Nutritional disorders				
Anorexia	19	2	14	<1
Nervous system disorders				
Peripheral Neuropathy[1]	42	7	45	11
Neuralgia	17	3	20	4
Paresthesia/dysesthesia	13	<1	10	0
Respiratory, thoracic and mediastinal disorders				
Cough	18	0	12	0
Skin and subcutaneous tissue disorders				
Rash[2]	22	1	18	1
Hand-foot syndrome	19	6	<1	0

[1] Peripheral neuropathy includes the following adverse reactions: peripheral sensory neuropathy, neuropathy peripheral, polyneuropathy, peripheral motor neuropathy, and neuropathy NOS.

[2] Rash includes the following adverse reactions: rash, rash erythematous, rash macular, rash maculo-papular, rash pruritic, exfoliative rash, and rash generalized.

6.2 Postmarketing Experience

The following additional adverse reactions have been identified during post approval use of DOXIL. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

Musculoskeletal and Connective Tissue Disorders: muscle spasms

Respiratory, Thoracic and Mediastinal Disorders: pulmonary embolism (in some cases fatal)

Hematologic disorders: Secondary acute myelogenous leukemia

Skin and subcutaneous tissue disorders: erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis

Secondary oral neoplasms: [see Warnings and Precautions (5.4)].

7 DRUG INTERACTIONS

No formal drug interaction studies have been conducted with DOXIL.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

Based on findings in animals, DOXIL can cause fetal harm when administered to a pregnant woman. In animal reproduction studies, DOXIL was embryotoxic in rats and abortifacient in rabbits following intravenous administration during organogenesis at doses approximately 0.12 times the recommended clinical dose *[see Data]*. There are no available human data informing the drug-associated risk. Advise pregnant women of the potential risk to a fetus.

The background risk of major birth defects and miscarriage for the indicated populations are unknown. However, the background risk in the U.S. general population of major birth defects is 2-4% and of miscarriage is 15-20% of clinically recognized pregnancies.

Data

Animal Data

DOXIL was embryotoxic at doses of 1 mg/kg/day in rats and was embryotoxic and abortifacient at 0.5 mg/kg/day in rabbits (both doses are about 0.12 times the recommended dose of 50 mg/m^2 human dose on a mg/m^2 basis). Embryotoxicity was characterized by increased embryo-fetal deaths and reduced live litter sizes.

8.2 Lactation

Risk Summary

It is not known whether DOXIL is present in human milk. Because many drugs, including anthracyclines, are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from DOXIL, discontinue breastfeeding during treatment with DOXIL.

8.3 Females and Males of Reproductive Potential

Contraception

Females

DOXIL can cause fetal harm when administered to a pregnant woman *[see Use in Specific Populations (8.1)]*. Advise females of reproductive potential to use effective contraception during and for 6 months after treatment with DOXIL.

Males

DOXIL may damage spermatozoa and testicular tissue, resulting in possible genetic fetal abnormalities. Males with female sexual partners of reproductive potential should use effective contraception during and for 6 months after treatment with DOXIL *[see Nonclinical Toxicology (13.1)]*.

Infertility

Females

In females of reproductive potential, DOXIL may cause infertility and result in amenorrhea. Premature menopause can occur with doxorubicin HCl. Recovery of menses and ovulation is related to age at treatment.

Males

DOXIL may result in oligospermia, azoospermia, and permanent loss of fertility. Sperm counts have been reported to return to normal levels in some men. This may occur several years after the end of therapy *[see Nonclinical Toxicology (13.1)]*.

8.4 Pediatric Use

The safety and effectiveness of DOXIL in pediatric patients have not been established.

8.5 Geriatric Use

Clinical studies of DOXIL conducted in patients with either epithelial ovarian cancer (Trial 4) or with AIDS-related Kaposi's sarcoma (Trial 5) did not contain sufficient numbers of patients aged 65 and over to determine whether they respond differently from younger subjects.

In Trial 6, of 318 patients treated with DOXIL in combination with bortezomib for multiple myeloma, 37% were 65 years of age or older and 8% were 75 years of age or older. No overall differences in safety or efficacy were observed between these patients and younger patients.

8.6 Hepatic Impairment

The pharmacokinetics of DOXIL has not been adequately evaluated in patients with hepatic impairment. Doxorubicin is eliminated in large part by the liver. Reduce DOXIL for serum bilirubin of 1.2 mg/dL or higher.

10 OVERDOSAGE

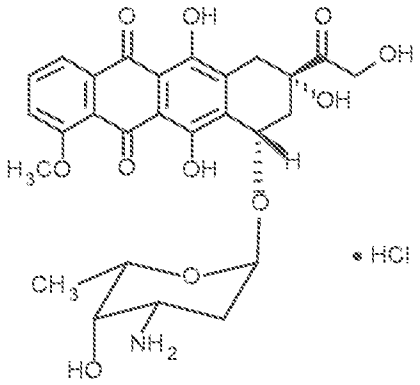
Acute overdosage with doxorubicin HCl causes increased risk of severe mucositis, leukopenia, and thrombocytopenia.

11 DESCRIPTION

DOXIL (doxorubicin HCl liposome injection) is doxorubicin hydrochloride (HCl), an anthracycline topoisomerase II inhibitor, that is encapsulated in STEALTH® liposomes for intravenous use.

The chemical name of doxorubicin HCl is (8S,10S)-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-8-glycolyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride. The molecular formula is C27-H29 -NO11•HCl; its molecular weight is 579.99.

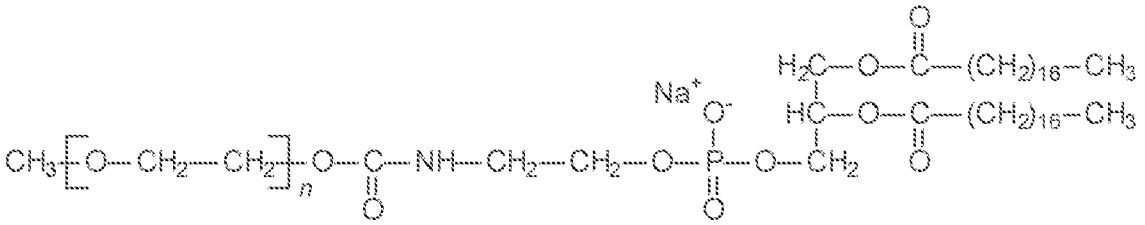
The molecular structure is:



DOXIL is a sterile, translucent, red liposomal dispersion in 10-mL or 30-mL glass, single use vials. Each vial contains 20 mg or 50 mg doxorubicin HCl at a concentration of 2 mg/mL and a pH of 6.5. The STEALTH liposome carriers are composed of cholesterol, 3.19 mg/mL; fully hydrogenated soy phosphatidylcholine (HSPC), 9.58 mg/mL; and N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (MPEG-DSPE), 3.19 mg/mL. Each mL also contains ammonium sulfate, approximately 2 mg; histidine as a buffer; hydrochloric acid and/or

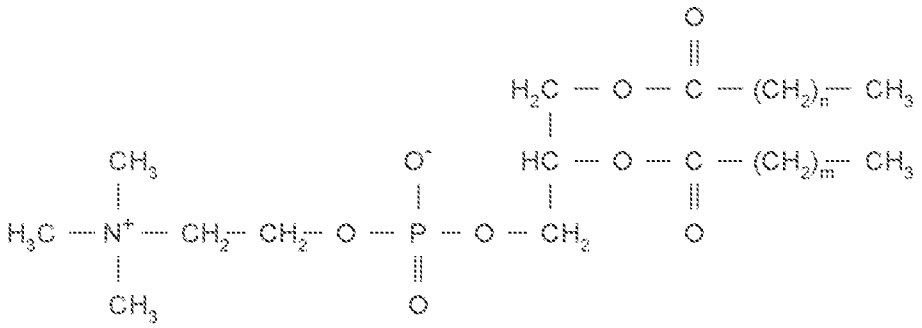
sodium hydroxide for pH control; and sucrose to maintain isotonicity. Greater than 90% of the drug is encapsulated in the STEALTH liposomes.

MPEG-DSPE has the following structural formula:



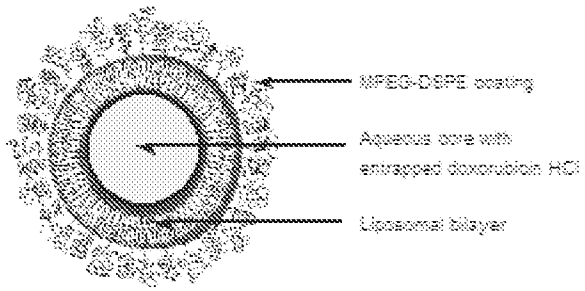
n = ca. 45

HSPC has the following structural formula:



m, n = 14 or 16

Representation of a STEALTH liposome:



12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

The active ingredient of DOXIL is doxorubicin HCl. The mechanism of action of doxorubicin HCl is thought to be related to its ability to bind DNA and inhibit nucleic acid synthesis. Cell structure studies have demonstrated rapid cell penetration and perinuclear

chromatin binding, rapid inhibition of mitotic activity and nucleic acid synthesis, and induction of mutagenesis and chromosomal aberrations.

12.3 Pharmacokinetics

The pharmacokinetic parameters for total doxorubicin following a single dose of DOXIL infused over 30 minutes are presented in Table 8.

Table 8: Pharmacokinetic Parameters of Total Doxorubicin from DOXIL in Patients With AIDS-Related Kaposi's Sarcoma

	Dose	
Parameter (units)	10 mg/m^2	20 mg/m^2
Peak Plasma Concentration (μg/mL)	4.12 ± 0.215	8.34 ± 0.49
Plasma Clearance (L/h/m^2)	0.056 ± 0.01	0.041 ± 0.004
Steady State Volume of Distribution (L/m^2)	2.83 ± 0.145	2.72 ± 0.120
AUC (μg/mL•h)	277 ± 32.9	590 ± 58.7
First Phase (λ_1) Half-Life (h)	4.7 ± 1.1	5.2 ± 1.4
Second Phase (λ_2) Half-Life (h)	52.3 ± 5.6	55.0 ± 4.8

N=23
Mean ± Standard Error

DOXIL displayed linear pharmacokinetics over the range of 10 to 20 mg/m^2. Relative to DOXIL doses at or below 20 mg/m^2, the pharmacokinetics of total doxorubicin following a 50 mg/m^2 DOXIL dose are nonlinear. At this dose, the elimination half-life of DOXIL is longer and the clearance lower compared to a 20 mg/m^2 dose.

Distribution:

Direct measurement of liposomal doxorubicin shows that at least 90% of the drug (the assay used cannot quantify less than 5-10% free doxorubicin) remains liposome-encapsulated during circulation.

In contrast to doxorubicin, which displays a large volume of distribution (range 700 to 1100 L/m^2), the small steady state volume of distribution of liposomal doxorubicin suggests that DOXIL is largely confined to vascular fluid. Doxorubicin becomes available after the liposomes are extravasated. Plasma protein binding of DOXIL has not been determined; the plasma protein binding of doxorubicin is approximately 70%.

Metabolism:

Doxorubicinol, the major metabolite of doxorubicin, was detected at concentrations of 0.8 to 26.2 ng/mL in the plasma of patients who received 10 or 20 mg/m^2 DOXIL.

Elimination:

The plasma clearance of total doxorubicin from DOXIL was 0.041 L/h/m^2 at a dose of 20 mg/m^2. Following administration of doxorubicin HCl, the plasma clearance of doxorubicin is 24 to 35 L/h/m^2.

13 NON-CLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, and Impairment of Fertility

Mutagenicity or carcinogenicity studies have not been conducted with DOXIL, however doxorubicin was shown to be mutagenic in the *in vitro* Ames assay, and clastogenic in multiple *in vitro* assays (CHO cell, V79 hamster cell, human lymphoblast, and SCE assays) and the *in vivo* mouse micronucleus assay. The possible adverse effects on fertility in animals have not been adequately evaluated. DOXIL resulted in mild to moderate ovarian and testicular atrophy in mice after administration of a single dose of 36 mg/kg (about 2 times the 50 mg/m^2 human dose on a mg/m^2 basis). Decreased testicular weights and hypospermia were observed in rats after repeat doses ≥ 0.25 mg/kg/day (about 0.03 times the 50 mg/m^2 human dose on a mg/m^2 basis), and diffuse degeneration of the seminiferous tubules and a marked decrease in spermatogenesis were observed in dogs after repeat doses of 1 mg/kg/day (about 0.4 times the 50 mg/m^2 human dose on a mg/m^2 basis).

14 CLINICAL STUDIES

14.1 Ovarian Cancer

DOXIL was studied in three open-label, single-arm, clinical studies of 176 patients with metastatic ovarian cancer (Trials 1, 2, and 3). One hundred forty-five of these patients were refractory to both paclitaxel- and platinum-based chemotherapy regimens, defined as disease progression while on treatment or relapse within 6 months of completing treatment. Patients received DOXIL at 50 mg/m^2 every 3 or 4 weeks for 3-6+ cycles in the absence of dose-limiting toxicity or disease progression.

The median age at diagnosis ranged from 52 to 64 years in the 3 studies, and the range was 22 to 85. Most patients had International Federation of Obstetricians and Gynecologists (FIGO) stage III or IV disease (ranging from 83% to 93%). Approximately one third of the patients had three or more prior lines of therapy (ranging from 22% to 33%).

The primary outcome measure was confirmed response rate based on Southwestern Oncology Group (SWOG) criteria for patients refractory to both paclitaxel- and a platinum-containing regimen. Secondary efficacy parameters were time to response, duration of response, and time to progression.

The response rates for the individual single arm trials are given in Table 9 below.

Table 9: Response Rates in Patients With Refractory Ovarian Cancer From Single Arm Ovarian Cancer Trials

	Trial 1 (U.S.) N=27	Trial 2 (U.S.) N=82	Trial 3 (non-U.S.) N=36
Response Rate	22.2%	17.1%	0%
95% Confidence Interval	8.6% - 42.3%	9.7% - 27.0%	0.0% - 9.7%

In a pooled analysis of Trials 1-3, the response rate for all patients refractory to paclitaxel and platinum agents was 13.8% (95% CI 8.1% to 19.3%). The median time to progression was 15.9 weeks, the median time to response was 17.6 weeks, and the duration of response was 39.4 weeks.

In Trial 4, a randomized, multicenter, open-label, trial in 474 patients with epithelial ovarian cancer after platinum-based chemotherapy, patients were randomized to receive either DOXIL 50 mg/m^2 every 4 weeks (n=239) or topotecan 1.5 mg/m^2 daily for 5 consecutive days every 3 weeks (n=235). Patients were stratified according to platinum sensitivity (response to initial platinum-based therapy and a progression-free interval of greater than 6 months off treatment) and the presence of bulky disease (tumor mass greater than 5 cm in size). The primary outcome measure was time to progression (TTP). Other endpoints included overall survival and objective response rate.

Of the 474 patients, the median age at diagnosis was 60 years (range 25 to 87), 90% were FIGO stage III and IV; 46% were platinum sensitive; and 45% had bulky disease.

There was no statistically significant difference in TTP between the two arms. Results are provided in Table 10.

Table 10: Results of Efficacy Analyses[1]

	Protocol Defined ITT Population	
	DOXIL (n=239)	Topotecan (n=235)
TTP (Protocol Specified Primary Endpoint)		
Median (Months)[2]	4.1	4.2
p-value[3]	0.62	
Hazard Ratio[4]	0.96	
95% CI for Hazard Ratio	(0.76, 1.20)	
Overall Survival		
Median (Months)[2]	14.4	13.7
p-value[5]	0.05	
Hazard Ratio[4]	0.82	
95% CI for Hazard Ratio	(0.68, 1.00)	
Response Rate		
Overall Response n (%)	47 (19.7)	40 (17.0)
Complete Response n (%)	9 (3.8)	11 (4.7)
Partial Response n (%)	38 (15.9)	29 (12.3)
Median Duration of Response (Months)[2]	6.9	5.9

[1] Analysis based on investigators' strata for protocol defined ITT population.
[2] Kaplan-Meier estimates.
[3] p-value is based on the stratified log-rank test.
[4] Hazard ratio is based on Cox proportional-hazard model with the treatment as single independent variable. A hazard ratio less than 1 indicates an advantage for DOXIL.
[5] p-value not adjusted for multiple comparisons.

14.2 AIDS-Related Kaposi's Sarcoma

DOXIL was studied in an open-label, single-arm, multicenter study at a dose of 20 mg/m^2 every 3 weeks, until disease progression or unacceptable toxicity (Trial 5).

Data is described for a cohort of 77 patients retrospectively identified as having disease progression on prior systemic combination chemotherapy (at least two cycles of a regimen containing at least two of three treatments: bleomycin, vincristine or vinblastine, or doxorubicin) or as being intolerant to such therapy. Forty-nine of the 77 (64%) patients had received prior doxorubicin HCl.

The median time on study was 5.1 months (range 1 day to 15 months). The median cumulative dose of DOXIL was 154 mg/m^2 (range 20 to 620 mg/m^2). Among the 77 patients, mean age was 38 years (range 24 to 54); 87% were Caucasian, 5% Hispanic, 4% Black, and 4% Asian/Other/Unknown; median CD4 count was 10 cells/mm^3; ACTG staging criteria were 78% poor risk for tumor burden, 96% poor risk for immune system, and 58% poor risk for systemic illness at baseline; and mean Karnofsky status score was 74%. All patients had cutaneous or subcutaneous lesions, 40% also had oral lesions, 26% pulmonary lesions, and 14% had lesions of the stomach/intestine.

Two analyses of tumor response were used: one based on investigator assessment of changes in lesions based on modified ACTG criteria (partial response defined as no new lesions, sites of disease, or worsening edema; flattening of ≥50% of previously raised lesions or area of indicator lesions decreasing by ≥50%; and response lasting at least 21 days with no prior progression), and one based on changes in up to five prospectively indentified representative indicator lesions (partial response defined as flattening of ≥50% of previously raised indicator lesions, or >50% decrease in the area of indicator lesions and lasting at least 21 days with no prior progression).

Of the 77 patients, 34 were evaluable for investigator assessment and 42 were evaluable for indicator lesion assessment; analyses of tumor responses are shown in Table 11.

Table 11: Response in Patients with Refractory[1] AIDS-Related Kaposi's Sarcoma

Investigator Assessment	All Evaluable Patients (n=34)	Evaluable Patients Who Received Prior Doxorubicin (n=20)
Response[2]		
Partial (PR)	27%	30%
Stable	29%	40%
Progression	44%	30%
Duration of PR (Days)		
Median	73	89
Range	42+ - 210+	42+ - 210+
Time to PR (Days)		
Median	43	53
Range	15 – 133	15 – 109
Indicator Lesion Assessment	**All Evaluable Patients (n=42)**	**Evaluable Patients Who Received Prior Doxorubicin (n=23)**
Response[2]		
Partial (PR)	48%	52%
Stable	26%	30%
Progression	26%	17%
Duration of PR (Days)		
Median	71	79
Range	22+ - 210+	35 - 210+
Time to PR (Days)		
Median	22	48
Range	15 – 109	15 – 109

[1] Patients with disease that progressed on prior combination chemotherapy or who were intolerant to such therapy.

[2] There were no complete responses in this population.

Retrospective efficacy analyses were performed in two trials that had subsets of patients who received single-agent DOXIL and who were on stable antiretroviral therapy for at least 60 days prior to enrollment and until a response was demonstrated. In one trial, 7 of 17 (40%) patients had a durable response (median duration not reached but was longer than 11.6 months). In the second trial, 4 of 11 patients (40%) on a stable antiretroviral therapy demonstrated durable responses.

14.3 Multiple Myeloma

The efficacy of DOXIL in combination with bortezomib was evaluated in Trial 6, a randomized, open-label, international, multicenter study in 646 patients who had not previously received bortezomib and whose disease progressed during or after at least one prior therapy. Patients were randomized (1:1) to receive either DOXIL (30 mg/m^2) administered IV on day 4 following bortezomib (1.3 mg/m^2 IV on days 1, 4, 8 and 11) or bortezomib alone every 3 weeks for up to 8 cycles or until disease progression or unacceptable toxicity. Patients who maintained a response were allowed to receive further treatment. The median number of cycles in each treatment arm was 5 (range 1-18).

The baseline demographics and clinical characteristics of the patients with multiple myeloma were similar between treatment arms (Table 12).

Table 12: Summary of Baseline Patient and Disease Characteristics

Patient Characteristics	DOXIL + bortezomib n=324	bortezomib n=322
Median age in years (range)	61 (28, 85)	62 (34, 88)
% Male/female	58 / 42	54 / 46
% Caucasian/Black/other	90 / 6/ 4	94 / 4 / 2
Disease Characteristics		
% with IgG/IgA/Light chain	57 / 27 / 12	62 / 24 /11
% β_2-microglobulin group		
≤2.5 mg/L	14	14
>2.5 mg/L and ≤5.5 mg/L	56	55
>5.5 mg/L	30	31
Serum M-protein (g/dL): Median (Range)	2.5 (0-10.0)	2.7 (0-10.0)
Urine M-protein (mg/24 hours): Median (Range)	107 (0-24883)	66 (0-39657)
Median Months Since Diagnosis	35.2	37.5
% Prior Therapy		
One	34	34
More than one	66	66
Prior Systemic Therapies for Multiple Myeloma		
Corticosteroid (%)	99	>99
Anthracyclines	68	67
Alkylating agent (%)	92	90
Thalidomide/lenalidomide (%)	40	43
Stem cell transplantation (%)	57	54

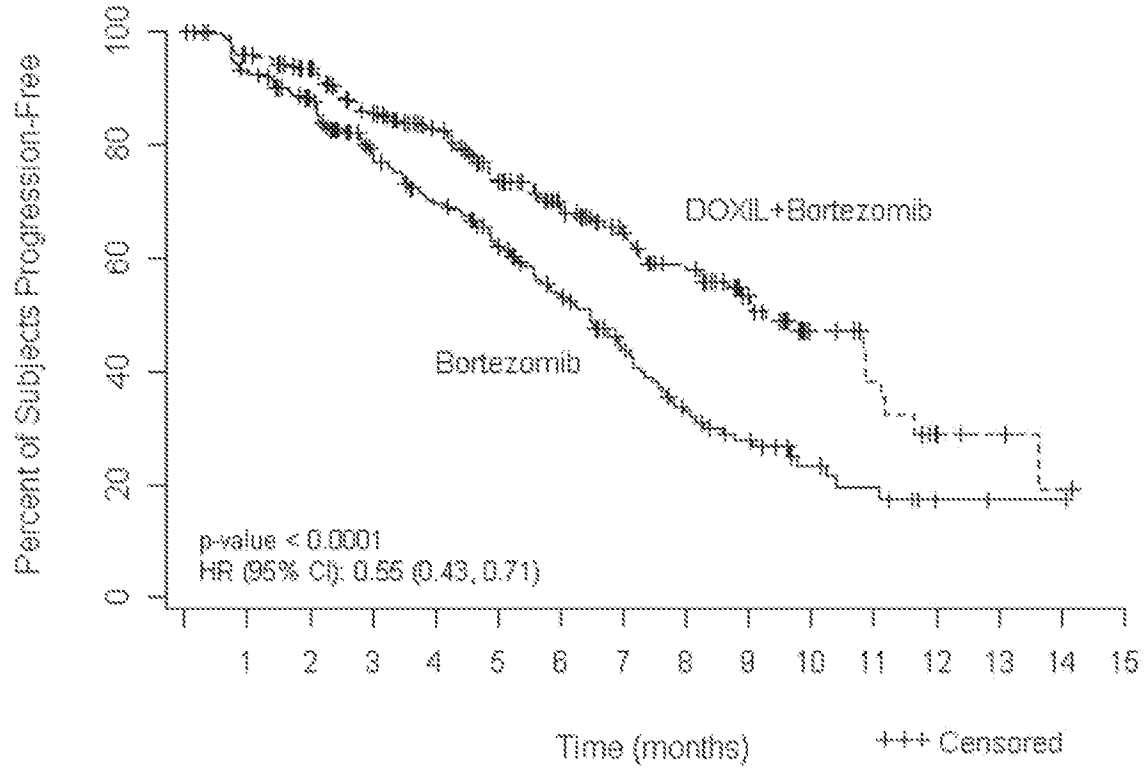
The primary outcome measure was time to progression (TTP). TTP was defined as the time from randomization to the first occurrence of progressive disease or death due to progressive disease. The combination arm demonstrated significant improvement in TTP. As the prespecified primary objective was achieved at the interim analysis, patients in the bortezomib monotherapy group were then allowed to receive the DOXIL + bortezomib combination. Efficacy results are as shown in Table 13 and Figure 1.

Table 13: Efficacy of DOXIL in Combination With Bortezomib in the Treatment of Patients With Multiple Myeloma

Endpoint	DOXIL + bortezomib n=324		Bortezomib n=322
Time to Progression[1]			
Progression or death due to progression (n)	99		150
Censored (n)	225		172
Median in days (months)	282 (9.3)		197 (6.5)
95% CI	250;338		170;217
Hazard ratio[2]		0.55	
(95% CI)		(0.43, 0.71)	
p-value[3]		<0.001	
Response (n)[4]	303		310
% Complete Response (CR)	5		3
% Partial Response (PR)	43		40
% CR + PR	48		43
p-value[5]		0.25	
Median Duration of Response (months)	10.2		7.0
(95% CI)	(10.2;12.9)		(5.9;8.3)

[1] Kaplan Meier estimate.
[2] Hazard ratio based on stratified Cox proportional hazards regression. A hazard ratio < 1 indicates an advantage for DOXIL+bortezomib.
[3] Stratified log-rank test.
[4] RR as per EBMT criteria.
[5] Cochran-Mantel-Haenszel test adjusted for the stratification factors.

Figure 1- Time to Progression Kaplan-Meier Curve



Number of Subjects at Risk

DOXIL+Bortezomib	324	301	269	201	170	127	97	70	56	38	19	13	6	4	2	0
Bortezomib	322	290	253	189	150	112	84	58	35	25	14	9	2	1	1	0

At the final analysis of survival, 78% of subjects in the DOXIL and bortezomib combination therapy group and 80% of subjects in the bortezomib monotherapy group had died after a median follow up of 8.6 years. The median survival was 33 months in the DOXIL and bortezomib combination therapy group and 31 months in the bortezomib monotherapy group. There was no difference observed in overall survival at the final analysis [HR for DOXIL + bortezomib vs. bortezomib= 0.96 (95% CI 0.80, 1.14)].

Seventy-eight percent of subjects in the DOXIL and bortezomib combination therapy group and 80% of subjects in the bortezomib monotherapy group had received subsequent therapy.

15 REFERENCES

1. "Hazardous Drugs", *OSHA*, http://www.osha.gov/SLTC/hazardousdrugs/index.html

16 HOW SUPPLIED/STORAGE AND HANDLING

DOXIL is a sterile, translucent, red liposomal dispersion in 10-mL or 30-mL glass, single use vials.

Each 10-mL vial contains 20 mg doxorubicin HCl at a concentration of 2 mg/mL.

Each 30-mL vial contains 50 mg doxorubicin HCl at a concentration of 2 mg/mL.

The following individually cartoned vials are available:

Table 14

mg in vial	fill volume	vial size	NDC #s
20 mg vial	10-mL	10-mL	59676-960-01
50 mg vial	25-mL	30-mL	59676-960-02

Refrigerate unopened vials of DOXIL at 2°- 8°C (36°- 46°F). Do not freeze.

DOXIL is a cytotoxic drug. Follow applicable special handling and disposal procedures.[1]

17 PATIENT COUNSELING INFORMATION

Cardiomyopathy

Advise patients to contact their healthcare provider if they develop symptoms of heart failure *[see Warnings and Precautions (5.1)]*.

Infusion-Related Reactions

Advise patients about the symptoms of infusion related reactions and to seek immediate medical attention if they develop any of these symptoms *[see Warnings and Precautions (5.2)]*.

Myelosuppression

Advise patients to contact their healthcare provider for a new onset fever or symptoms of infection.

Hand-Foot Syndrome

Advise patients to notify their healthcare provider if they experience tingling or burning, redness, flaking, bothersome swelling, small blisters, or small sores on the palms of their hands or soles of their feet (symptoms of Hand-Foot Syndrome) *[see Warnings and Precautions (5.3)]*.

Stomatitis

Advise patients to notify their healthcare provider if they develop painful redness, swelling, or sores in the mouth (symptoms of stomatitis).

Embryofetal Toxicity

Advise females of reproductive potential of the potential risk to a fetus and to inform their healthcare provider with a known or suspected pregnancy *[see Warnings and Precautions (5.5) and Use in Specific Populations (8.1)]*.

Advise females and males of reproductive potential to use effective contraception during and for 6 months following treatment with DOXIL *[see Use in Specific Populations (8.3)]*.

Lactation

Advise females not to breastfeed during treatment with DOXIL *[see Use in Specific Populations (8.2)]*.

Infertility

Advise females and males of reproductive potential that DOXIL may cause temporary or permanent infertility *[see Use in Specific Populations (8.3)]*.

Discoloration of Urine and Body Fluids

Inform patients that following DOXIL administration, a reddish-orange color to the urine and other body fluids may be observed. This nontoxic reaction is due to the color of the product and will dissipate as the drug is eliminated from the body.

Manufactured by:
ALZA Corporation
Bedford, OH 44146

or

TTY Biopharm Company Limited
No. 838, Sec. 1, Chung Hwa Rd.
Chung-Li, Taoyuan, Taiwan, R.O.C.

Manufactured for:
Janssen Products, LP
Horsham, PA 19044

An ALZA STEALTH®

Technology Product

STEALTH® and DOXIL® are registered trademarks of ALZA Corporation.

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use DOXIL safely and effectively. See full prescribing information for DOXIL.

DOXIL® (doxorubicin HCl liposome injection) for intravenous infusion
Initial U.S. Approval: 1995

WARNING: INFUSION REACTIONS, MYELOSUPPRESSION, CARDIOTOXICITY, LIVER IMPAIRMENT, SUBSTITUTION

See full prescribing information for complete boxed warning.

- **Myocardial damage may lead to congestive heart failure and may occur as the total cumulative dose of doxorubicin HCl approaches 550 mg/m^2. Cardiac toxicity may also occur at lower cumulative doses with mediastinal irradiation or concurrent cardiotoxic agents (5.1).**
- **Acute infusion-related reactions, sometimes reversible upon terminating or slowing infusion, occurred in up to 10% of patients. Serious and sometimes fatal allergic/anaphylactoid-like infusion reactions have been reported. Medications/emergency equipment to treat such reactions should be available for immediate use (5.2).**
- **Severe myelosuppression may occur (5.3)**
- **Reduce dosage in patients with impaired hepatic function (2.6).**
- **Accidental substitution of DOXIL resulted in severe side effects. Do not substitute on mg per mg basis with doxorubicin HCl (2.1).**

----------RECENT MAJOR CHANGES----------

Contraindications, Nursing Mothers (4)	Removed 9/2012
Warnings and Precautions, Secondary Oral Neoplasms (5.9)	8/2013

----------INDICATIONS AND USAGE----------

DOXIL is an anthracycline topoisomerase inhibitor indicated for:

- **Ovarian cancer (1.1)**

After failure of platinum-based chemotherapy.

- **AIDS-related Kaposi's Sarcoma (1.2)**

After failure of prior systemic chemotherapy or intolerance to such therapy.

- **Multiple Myeloma (1.3)**

In combination with bortezomib in patients who have not previously received bortezomib and have received at least one prior therapy.

----------DOSAGE AND ADMINISTRATION----------

Administer DOXIL at an initial rate of 1 mg/min to minimize the risk of infusion reactions. If no infusion related reactions occur, increase rate of infusion to complete administration over 1 hour. Do not administer as bolus injection or undiluted solution (2.1).

- **Ovarian cancer:** 50 mg/m^2 IV every 4 weeks for 4 courses minimum (2.2)
- **AIDS-related Kaposi's Sarcoma:** 20 mg/m^2 IV every 3 weeks (2.3)
- **Multiple Myeloma:** 30 mg/m^2 IV on day 4 following bortezomib which is administered at 1.3 mg/m^2 bolus on days 1, 4, 8 and 11, every 3 weeks (2.4)

----------DOSAGE FORMS AND STRENGTHS----------

Single use vial: 20 mg/10 mL and 50 mg/25mL (3)

----------CONTRAINDICATIONS----------

- Hypersensitivity reactions to a conventional formulation of doxorubicin HCl or the components of DOXIL (4, 5.2)

----------WARNINGS AND PRECAUTIONS----------

- Hand-Foot Syndrome may occur. Dose modification or discontinuation may be required (5.4)
- Radiation recall reaction may occur (5.5)

----------ADVERSE REACTIONS----------

Most common adverse reactions (>20%) are asthenia, fatigue, fever, anorexia, nausea, vomiting, stomatitis, diarrhea, constipation, hand and foot syndrome, rash, neutropenia, thrombocytopenia and anemia (6).

To report SUSPECTED ADVERSE REACTIONS contact Janssen Products, LP at 1-800-JANSSEN (1-800-526-7736) or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

----------DRUG INTERACTIONS----------

- DOXIL may interact with drugs known to interact with conventional formulations of Doxorubicin HCl. (7)

----------USE IN SPECIFIC POPULATIONS----------

- DOXIL can cause fetal harm when used during pregnancy. (5.6, 8.1)
- Discontinue nursing during treatment with DOXIL (8.3).

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 08/2013

FULL PRESCRIBING INFORMATION: CONTENTS*

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- 1.3 Multiple Myeloma

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17 PATIENT COUNSELING INFORMATION

*Sections or subsections omitted from the full prescribing information are not listed

FULL PRESCRIBING INFORMATION

WARNING: INFUSION REACTIONS, MYELOSUPPRESSION, CARDIOTOXICITY, LIVER IMPAIRMENT, ACCIDENTAL SUBSTITUTION

1. **The use of DOXIL (doxorubicin HCl liposome injection) may lead to cardiac toxicity. Myocardial damage may lead to congestive heart failure and may occur as the total cumulative dose of doxorubicin HCl approaches 550 mg/m^2. In a clinical study in patients with advanced breast cancer, 250 patients received DOXIL at a starting dose of 50 mg/m^2 every 4 weeks. At all cumulative anthracycline doses between 450-500 mg/m^2 or between 500-550 mg/m^2, the risk of cardiac toxicity for patients treated with DOXIL was 11%. Prior use of other anthracyclines or anthracenediones should be included in calculations of total cumulative dosage. Cardiac toxicity may also occur at lower cumulative doses in patients with prior mediastinal irradiation or who are receiving concurrent cyclophosphamide therapy *[see Warnings and Precautions (5.1)]*.**

2. **Acute infusion-related reactions including, but not limited to, flushing, shortness of breath, facial swelling, headache, chills, back pain, tightness in the chest or throat, and/or hypotension have occurred in up to 10% of patients treated with DOXIL. In most patients, these reactions resolve over the course of several hours to a day once the infusion is terminated. In some patients, the reaction has resolved with slowing of the infusion rate. Serious and sometimes life-threatening or fatal allergic/anaphylactoid-like infusion reactions have been reported. Medications to treat such reactions, as well as emergency equipment, should be available for immediate use. DOXIL should be administered at an initial rate of 1 mg/min to minimize the risk of infusion reactions *[see Warnings and Precautions (5.2)]*.**

3. **Severe myelosuppression may occur *[see Warnings and Precautions (5.3)]*.**

4. **Dosage should be reduced in patients with impaired hepatic function *[see Dosage and Administration (2.6) and Use in Specific Populations (8.6)]*.**

5. **Accidental substitution of DOXIL for doxorubicin HCl has resulted in severe side effects. DOXIL should not be substituted for doxorubicin HCl on a mg per mg basis *[see Dosage and Administration (2.1)]*.**

1 INDICATIONS AND USAGE

1.1 Ovarian Cancer

DOXIL (doxorubicin HCl liposome injection) is indicated for the treatment of patients with ovarian cancer whose disease has progressed or recurred after platinum-based chemotherapy.

1.2 AIDS-Related Kaposi's Sarcoma

DOXIL is indicated for the treatment of AIDS-related Kaposi's sarcoma in patients after failure of prior systemic chemotherapy or intolerance to such therapy.

1.3 Multiple Myeloma

DOXIL in combination with bortezomib is indicated for the treatment of patients with multiple myeloma who have not previously received bortezomib and have received at least one prior therapy.

2 DOSAGE AND ADMINISTRATION

2.1 Usage and Administration Precautions

Liposomal encapsulation can substantially affect a drug's functional properties relative to those of the unencapsulated drug. Therefore DO NOT SUBSTITUTE one drug for the other.

Do not administer as a bolus injection or an undiluted solution. Rapid infusion may increase the risk of infusion-related reactions *[see Warnings and Precautions (5.2)]*. DOXIL must not be given by the intramuscular or subcutaneous route.

Until specific compatibility data are available, it is not recommended that DOXIL be mixed with other drugs.

DOXIL should be considered an irritant and precautions should be taken to avoid extravasation. With intravenous administration of DOXIL, extravasation may occur with or without an accompanying stinging or burning sensation, even if blood returns well on aspiration of the infusion needle. If any signs or symptoms of extravasation have occurred, the infusion should be immediately terminated and restarted in another vein. The application of ice over the site of extravasation for approximately 30 minutes may be helpful in alleviating the local reaction.

2.2 Patients With Ovarian Cancer

DOXIL (doxorubicin HCl liposome injection) should be administered intravenously at a dose of 50 mg/m^2 (doxorubicin HCl equivalent) at an initial rate of 1 mg/min to minimize the risk of infusion reactions. If no infusion-related adverse reactions are observed, the rate of infusion can be increased to complete administration of the drug over one hour. The patient should be dosed once every 4 weeks, for as long as the patient does not progress, shows no evidence of cardiotoxicity *[see Warnings and Precautions (5.1)]*, and continues to tolerate treatment. A minimum of 4 courses is recommended because median time to response in clinical trials was 4 months. To manage adverse reactions such as hand-foot syndrome (HFS), stomatitis, or hematologic toxicity the doses may be delayed or reduced *[see Dosage and Administration (2.5)]*. Pretreatment with or concomitant use of antiemetics should be considered.

2.3 Patients With AIDS-Related Kaposi's Sarcoma

DOXIL (doxorubicin HCl liposome injection) should be administered intravenously at a dose of 20 mg/m^2 (doxorubicin HCl equivalent). An initial rate of 1 mg/min should be used to minimize the risk of infusion-related reactions. If no infusion-related adverse reactions are observed, the infusion rate should be increased to complete the administration of the drug over one hour. The dose should be repeated once every three weeks, for as long as patients respond satisfactorily and tolerate treatment.

2.4 Patients With Multiple Myeloma

Bortezomib is administered at a dose of 1.3 mg/m^2 as intravenous bolus on days 1, 4 , 8 and 11, every three weeks. DOXIL 30 mg/m^2 should be administered as a 1-hr intravenous infusion on day 4 following bortezomib. With the first DOXIL dose, an initial rate of 1 mg/min should be used to minimize the risk of infusion-related reactions. If no infusion-related adverse reactions are observed, the infusion rate should be increased to complete the administration of the drug over one hour. Patients may be treated for up to 8 cycles until disease progression or the occurrence of unacceptable toxicity.

2.5 Dose Modification Guidelines

DOXIL exhibits nonlinear pharmacokinetics at 50 mg/m^2, therefore, dose adjustments may result in a non-proportional greater change in plasma concentration and exposure to the drug *[see Clinical Pharmacology (12.3)]*.

Patients should be carefully monitored for toxicity. Adverse reactions, such as HFS, hematologic toxicities, and stomatitis may be managed by dose delays and adjustments. Following the first appearance of a Grade 2 or higher adverse reactions, the dosing should be adjusted or delayed as described in the following tables. Once the dose has been reduced, it should not be increased at a later time.

Recommended Dose Modification Guidelines

Table 1: Hand-Foot Syndrome (HFS)

Toxicity Grade	Dose Adjustment
1 (mild erythema, swelling, or desquamation not interfering with daily activities)	**Redose unless patient has experienced previous Grade 3 or 4 HFS.** If so, delay up to 2 weeks and decrease dose by 25%. Return to original dose interval.
2 (erythema, desquamation, or swelling interfering with, but not precluding normal physical activities; small blisters or ulcerations less than 2 cm in diameter)	**Delay dosing up to 2 weeks or until resolved to Grade 0-1.** If after 2 weeks there is no resolution, DOXIL should be discontinued. If resolved to Grade 0-1 within 2 weeks, and there are no prior Grade 3-4 HFS, continue treatment at previous dose and return to original dose interval. If patient experienced previous Grade 3-4 toxicity, continue treatment with a 25% dose reduction and return to original dose interval.
3 (blistering, ulceration, or swelling interfering with walking or normal daily activities; cannot wear regular clothing)	**Delay dosing up to 2 weeks or until resolved to Grade 0-1.** Decrease dose by 25% and return to original dose interval. If after 2 weeks there is no resolution, DOXIL should be discontinued.
4 (diffuse or local process causing infectious complications, or a bed ridden state or hospitalization)	**Delay dosing up to 2 weeks or until resolved to Grade 0-1.** Decrease dose by 25% and return to original dose interval. If after 2 weeks there is no resolution, DOXIL should be discontinued.

Table 2: Hematological Toxicity

Grade	ANC	Platelets	Modification
1	1,500 – 1,900	75,000 - 150,000	Resume treatment with no dose reduction
2	1,000 - <1,500	50,000 - <75,000	Wait until ANC $\geq$ 1,500 and platelets $\geq$ 75,000; redose with no dose reduction
3	500 – 999	25,000 - <50,000	Wait until ANC $\geq$ 1,500 and platelets $\geq$ 75,000; redose with no dose reduction
4	<500	<25,000	Wait until ANC $\geq$ 1,500 and platelets $\geq$ 75,000; redose at 25% dose reduction or continue full dose with cytokine support

Table 3: Stomatitis

Toxicity Grade	Dose Adjustment
1 (painless ulcers, erythema, or mild soreness)	**Redose unless patient has experienced previous Grade 3 or 4 toxicity.** If so, delay up to 2 weeks and decrease dose by 25%. Return to original dose interval.
2 (painful erythema, edema, or ulcers, but can eat)	**Delay dosing up to 2 weeks or until resolved to Grade 0-1.** If after 2 weeks there is no resolution, DOXIL should be discontinued. If resolved to Grade 0-1 within 2 weeks and there was no prior Grade 3-4 stomatitis, continue treatment at previous dose and return to original dose interval. If patient experienced previous Grade 3-4 toxicity, continue treatment with a 25% dose reduction and return to original dose interval.
3 (painful erythema, edema, or ulcers, and cannot eat)	**Delay dosing up to 2 weeks or until resolved to Grade 0-1.** Decrease dose by 25% and return to original dose interval. If after 2 weeks there is no resolution, DOXIL should be discontinued.
4 (requires parenteral or enteral support)	**Delay dosing up to 2 weeks or until resolved to Grade 0-1.** Decrease dose by 25% and return to DOXIL original dose interval. If after 2 weeks there is no resolution, DOXIL should be discontinued.

Multiple Myeloma

For patients treated with DOXIL in combination with bortezomib who experience hand-foot syndrome or stomatitis, the DOXIL dose should be modified as described in Tables 1 and 3 above. Table 4 describes dosage adjustments for DOXIL and bortezomib combination therapy. For bortezomib dosing and dosage adjustments, see manufacturer's prescribing information.

Table 4: Dosage adjustments for DOXIL + bortezomib combination therapy

Patient status	DOXIL	bortezomib
Fever ≥38°C and ANC <1,000/mm^3	Do not dose this cycle if before Day 4; if after Day 4, reduce next dose by 25%.	Reduce next dose by 25%
On any day of drug administration after Day 1 of each cycle: Platelet count <25,000/mm^3 Hemoglobin <8g/dL ANC <500/mm^3	Do not dose this cycle if before Day 4; if after Day 4 reduce next dose by 25% in the following cycles if bortezomib is reduced for hematologic toxicity.	Do not dose; if 2 or more doses are not given in a cycle, reduce dose by 25% in following cycles.
Grade 3 or 4 non-hematologic drug related toxicity	Do not dose until recovered to Grade <2 and reduce dose by 25% for all subsequent doses.	Do not dose until recovered to Grade <2 and reduce dose by 25% for all subsequent doses.
Neuropathic pain or peripheral neuropathy	No dosage adjustments.	See bortezomib manufacturer's prescribing information for dosage adjustments in patients with neuropathic pain.

2.6 Patients With Impaired Hepatic Function

Limited clinical experience exists in treating patients with hepatic impairment with DOXIL. Based on experience with doxorubicin HCl, it is recommended that the DOXIL dosage be reduced if the bilirubin is elevated as follows: serum bilirubin 1.2 to 3.0 mg/dL - give ½ normal dose; serum bilirubin > 3 mg/dL - give ¼ normal dose.

No information, including dosage adjustments, is available for patients with multiple myeloma with hepatic impairment.

2.7 Preparation for Intravenous Administration

Each 10-mL vial contains 20 mg doxorubicin HCl at a concentration of 2 mg/mL.

Each 30-mL vial contains 50 mg doxorubicin HCl at a concentration of 2 mg/mL.

DOXIL doses up to 90 mg must be diluted in 250 mL of 5% Dextrose Injection, USP prior to administration. Doses exceeding 90 mg should be diluted in 500 mL of 5% Dextrose Injection, USP prior to administration. Aseptic technique must be strictly observed since no preservative or bacteriostatic agent is present in DOXIL. Diluted DOXIL should be refrigerated at 2°C to 8°C (36°F to 46°F) and administered within 24 hours.

Do not use with in-line filters.

Do not mix with other drugs.

Do not use with any diluent other than 5% Dextrose Injection.

Do not use any bacteriostatic agent, such as benzyl alcohol.

DOXIL is not a clear solution but a translucent, red liposomal dispersion.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use if a precipitate or foreign matter is present.

Rapid flushing of the infusion line should be avoided.

2.8 Procedure for Proper Handling and Disposal

Caution should be exercised in the handling and preparation of DOXIL.

The use of gloves is required.

If DOXIL comes into contact with skin or mucosa, immediately wash thoroughly with soap and water.

DOXIL should be considered an irritant and precautions should be taken to avoid extravasation. With intravenous administration of DOXIL, extravasation may occur with or without an accompanying stinging or burning sensation, even if blood returns well on aspiration of the infusion needle. If any signs or symptoms of extravasation have occurred, the infusion should be immediately terminated and restarted in another vein. **DOXIL must not be given by the intramuscular or subcutaneous route.**

DOXIL should be handled and disposed of in a manner consistent with other anticancer drugs. Several guidelines on this subject exist *[see References (15)]*.

3 DOSAGE FORMS AND STRENGTHS

- Single use vial: 20 mg/10 mL
- Single use vial: 50 mg/25 mL

4 CONTRAINDICATIONS

DOXIL (doxorubicin HCl liposome injection) is contraindicated in patients who have a history of hypersensitivity reactions to a conventional formulation of doxorubicin HCl or the components of DOXIL *[see Warnings and Precautions (5.2)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Cardiac Toxicity

Special attention must be given to the risk of myocardial damage from cumulative doses of doxorubicin HCl. Acute left ventricular failure may occur with doxorubicin, particularly in patients who have received a total cumulative dosage of doxorubicin exceeding the currently recommended limit of 550 mg/m^2. Lower (400 mg/m^2) doses appear to cause heart failure in patients who have received radiotherapy to the mediastinal area or concomitant therapy with other potentially cardiotoxic agents such as cyclophosphamide.

Prior use of other anthracyclines or anthracenodiones should be included in calculations of total cumulative dosage. Congestive heart failure or cardiomyopathy may be encountered after discontinuation of anthracycline therapy. Patients with a history of cardiovascular disease should be administered DOXIL only when the potential benefit of treatment outweighs the risk.

Cardiac function should be carefully monitored in patients treated with DOXIL. The most definitive test for anthracycline myocardial injury is endomyocardial biopsy. Other methods, such as echocardiography or multigated radionuclide scans, have been used to monitor cardiac function during anthracycline therapy. Any of these methods should be employed to monitor potential cardiac toxicity in patients treated with DOXIL. If these test results indicate possible cardiac injury associated with DOXIL therapy, the benefit of continued therapy must be carefully weighed against the risk of myocardial injury.

In a clinical study in patients with advanced breast cancer, 250 patients received DOXIL at starting dose of 50 mg/m^2 every 4 weeks. At all cumulative anthracycline doses between 450-500 mg/m^2, or between 500–550 mg/m^2, the risk of cardiac toxicity for patients treated with DOXIL was 11%. In this study, cardiotoxicity was defined as a decrease of >20% from baseline if the resting left ventricular ejection fraction (LVEF) remained in the normal range, or a decrease of >10% if the resting LVEF became abnormal (less than the institutional lower limit of normal). The data on left ventricular ejection fraction (LVEF) defined cardiotoxicity and congestive heart failure (CHF) are in the table below.

Table 5: Number of Patients With Advanced Breast Cancer

	DOXIL (n=250)
Patients who Developed Cardiotoxicity (LVEF Defined)	10
Cardiotoxicity (With Signs & Symptoms of CHF)	0
Cardiotoxicity (no Signs & Symptoms of CHF)	10
Patients With Signs and Symptoms of CHF Only	2

In the randomized multiple myeloma study, the incidence of heart failure events (ventricular dysfunction, cardiac failure, right ventricular failure, congestive cardiac failure, chronic cardiac failure, acute pulmonary edema and pulmonary edema) was similar in the DOXIL+bortezomib group and the bortezomib monotherapy group, 3% in each group. LVEF decrease was defined as an absolute decrease of ≥15% over baseline or a ≥5% decrease below the institutional lower limit of normal. Based on this definition, 25 patients in the bortezomib arm (8%) and 42 patients in the DOXIL + bortezomib arm (13%) experienced a reduction in LVEF.

5.2 Infusion Reactions

Acute infusion-related reactions were reported in 7.1% of patients treated with DOXIL in the randomized ovarian cancer study. These reactions were characterized by one or more of the following symptoms: flushing, shortness of breath, facial swelling, headache, chills, chest pain, back pain, tightness in the chest and throat, fever, tachycardia, pruritus, rash, cyanosis, syncope, bronchospasm, asthma, apnea, and hypotension. In most patients, these reactions resolve over the course of several hours to a day once the infusion is terminated. In some patients, the reaction resolved when the rate of infusion was slowed. In this study, two patients treated with DOXIL (0.8%) discontinued due to infusion-related reactions. In clinical studies, six patients with AIDS-related Kaposi's sarcoma (0.9%) and 13 (1.7%) solid tumor patients discontinued DOXIL therapy because of infusion-related reactions.

Serious and sometimes life-threatening or fatal allergic/anaphylactoid-like infusion reactions have been reported. Medications to treat such reactions, as well as emergency equipment, should be available for immediate use.

The majority of infusion-related events occurred during the first infusion. Similar reactions have not been reported with conventional doxorubicin and they presumably represent a reaction to the DOXIL liposomes or one of its surface components.

The initial rate of infusion should be 1 mg/min to help minimize the risk of infusion reactions *[see Dosage and Administration (2)]*.

5.3 Myelosuppression

Because of the potential for bone marrow suppression, careful hematologic monitoring is required during use of DOXIL, including white blood cell, neutrophil, platelet counts, and Hgb/Hct. With the recommended dosage schedule, leukopenia is usually transient. Hematologic toxicity may require dose reduction or delay or suspension of DOXIL therapy. Persistent severe myelosuppression may result in superinfection, neutropenic fever, or

hemorrhage. Development of sepsis in the setting of neutropenia has resulted in discontinuation of treatment and, in rare cases, death.

DOXIL may potentiate the toxicity of other anticancer therapies. In particular, hematologic toxicity may be more severe when DOXIL is administered in combination with other agents that cause bone marrow suppression.

In patients with relapsed ovarian cancer, myelosuppression was generally moderate and reversible. In the three single-arm studies, anemia was the most common hematologic adverse reaction (52.6%), followed by leukopenia (WBC< 4,000 mm^3; 42.2%), thrombocytopenia (24.2%), and neutropenia (ANC <1,000; 19.0%). In the randomized study, anemia was the most common hematologic adverse reaction (40.2%), followed by leukopenia (WBC <4,000 mm^3; 36.8%), neutropenia (ANC <1,000; 35.1%), and thrombocytopenia (13.0%) *[see Adverse Reactions (6.2)]*.

In patients with relapsed ovarian cancer, 4.6% received G-CSF (or GM-CSF) to support their blood counts *[see Dosage and Administration (2.5)]*.

For patients with AIDS-related Kaposi's sarcoma who often present with baseline myelosuppression due to such factors as their HIV disease or concomitant medications, myelosuppression appears to be the dose-limiting adverse reaction at the recommended dose of 20 mg/m^2 *[see Adverse Reactions (6.2)]*. Leukopenia is the most common adverse reaction experienced in this population; anemia and thrombocytopenia can also be expected. Sepsis occurred in 5% of patients; for 0.7% of patients the event was considered possibly or probably related to DOXIL. Eleven patients (1.6%) discontinued study because of bone marrow suppression or neutropenia.

Table 10 presents data on myelosuppression in patients with multiple myeloma receiving DOXIL and bortezomib in combination *[see Adverse Reactions (6.2)]*.

5.4 Hand-Foot Syndrome (HFS)

In the randomized ovarian cancer study, 50.6% of patients treated with DOXIL at 50 mg/m^2 every 4 weeks experienced HFS (developed palmar-plantar skin eruptions characterized by swelling, pain, erythema and, for some patients, desquamation of the skin on the hands and the feet), with 23.8% of the patients reporting HFS Grade 3 or 4 events. Ten subjects (4.2%) discontinued treatment due to HFS or other skin toxicity. HFS toxicity grades are described above *[see definitions of HFS grades in Dosage and Administration (2.5)]*.

Among 705 patients with AIDS-related Kaposi's sarcoma treated with DOXIL at 20 mg/m^2 every 2 weeks, 24 (3.4%) developed HFS, with 3 (0.9%) discontinuing.

In the randomized multiple myeloma study, 19% of patients treated with DOXIL at 30 mg/m^2 every three weeks experienced HFS.

HFS was generally observed after 2 or 3 cycles of treatment but may occur earlier. In most patients the reaction is mild and resolves in one to two weeks so that prolonged delay of therapy need not occur. However, dose modification may be required to manage HFS *[see Dosage and Administration (2.5)]*. The reaction can be severe and debilitating in some patients and may require discontinuation of treatment.

5.5 Radiation Recall Reaction

Recall reaction has occurred with DOXIL administration after radiotherapy.

5.6 Fetal Mortality

Pregnancy Category D

DOXIL can cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies in pregnant women. If DOXIL is to be used during pregnancy, or if the patient becomes pregnant during therapy, the patient should be apprised of the potential hazard to the fetus. If pregnancy occurs in the first few months following treatment with DOXIL, the prolonged half-life of the drug must be considered. Women of childbearing potential should be advised to avoid pregnancy during treatment with Doxil. *[see Use in Specific Populations (8.1)]*.

5.7 Toxicity Potentiation

The doxorubicin in DOXIL may potentiate the toxicity of other anticancer therapies. Exacerbation of cyclophosphamide-induced hemorrhagic cystitis and enhancement of the hepatotoxicity of 6-mercaptopurine have been reported with the conventional formulation of doxorubicin HCl. Radiation-induced toxicity to the myocardium, mucosae, skin, and liver have been reported to be increased by the administration of doxorubicin HCl.

5.8 Monitoring: Laboratory Tests

Complete blood counts, including platelet counts, should be obtained frequently and at a minimum prior to each dose of DOXIL *[see Warnings and Precautions (5.3)]*.

5.9 Secondary Oral Neoplasms

Secondary oral cancers, primarily squamous cell carcinoma, have been reported from post-marketing experience in patients with long-term (more than one year) exposure to DOXIL. These malignancies were diagnosed both during treatment with DOXIL and up to 6 years

after the last dose. Examine patients at regular intervals for the presence of oral ulceration or with any oral discomfort that may be indicative of secondary oral cancer.

The altered pharmacokinetics and preferential tissue distribution of liposomal doxorubicin that contributes to enhanced skin toxicity and mucositis compared to free doxorubicin may play a role in the development of oral secondary malignancies with long-term use.

6 ADVERSE REACTIONS

6.1 Overall Adverse Reactions Profile

The following adverse reactions are discussed in more detail in other sections of the labeling.

- Cardiac Toxicity *[see Warnings and Precautions (5.1)]*
- Infusion reactions *[see Warnings and Precautions (5.2)]*
- Myelosuppression *[see Warnings and Precautions (5.3)]*
- Hand-Foot syndrome *[see Warnings and Precautions (5.4)]*
- Secondary Oral Neoplasms *[see Warnings and Precautions (5.9)]*

The most common adverse reactions observed with DOXIL are asthenia, fatigue, fever, nausea, stomatitis, vomiting, diarrhea, constipation, anorexia, hand-foot syndrome, rash and neutropenia, thrombocytopenia and anemia.

The most common serious adverse reactions observed with DOXIL are described in Section 6.2.

The safety data described below reflect exposure to DOXIL in 1310 patients including: 239 patients with ovarian cancer, 753 patients with AIDS-related Kaposi's sarcoma and 318 patients with multiple myeloma *[see Adverse Reactions (6.2)]*.

6.2 Adverse Reactions in Clinical Trials

Because clinical trials are conducted under widely varying conditions, the adverse reaction rates observed cannot be directly compared to rates on other clinical trials and may not reflect the rates observed in clinical practice.

The following tables present adverse reactions from clinical trials of DOXIL in ovarian cancer and AIDS-Related Kaposi's sarcoma.

Patients With Ovarian Cancer

The safety data described below are from 239 patients with ovarian cancer treated with DOXIL (doxorubicin HCl liposome injection) at 50 mg/m^2 once every 4 weeks for a

minimum of 4 courses in a randomized, multicenter, open-label study. In this study, patients received DOXIL for a median number of 98.0 days (range 1-785 days). The population studied was 27-87 years of age, 91% Caucasian, 6% Black and 3% Hispanic and other.

Table 6 presents the hematologic adverse reactions from the randomized study of DOXIL compared to topotecan.

Table 6: Ovarian Cancer Randomized Study Hematology Data Reported in Patients With Ovarian Cancer

	DOXIL Patients (n = 239)	Topotecan Patients (n = 235)
Neutropenia		
500 - <1000/mm^3	19 (7.9%)	33 (14.0%)
<500/mm^3	10 (4.2%)	146 (62.1%)
Anemia		
6.5 - <8 g/dL	13 (5.4%)	59 (25.1%)
< 6.5 g/dL	1 (0.4%)	10 (4.3%)
Thrombocytopenia		
10,000 - <50,000/mm^3	3 (1.3%)	40 (17.0%)
<10,000/mm^3	0 (0.0%)	40 (17.0%)

Table 7 presents a comparative profile of the non-hematologic adverse reactions from the randomized study of DOXIL compared to topotecan.

Table 7: Ovarian Cancer Randomized Study

Non-Hematologic Adverse Reaction 10% or Greater	DOXIL (%) treated (n = 239)		Topotecan (%) treated (n =235)	
	All grades	Grades 3-4	All grades	Grades 3-4
Body as a Whole				
Asthenia	40.2	7.1	51.5	8.1
Fever	21.3	0.8	30.6	5.5
Mucous Membrane Disorder	14.2	3.8	3.4	0
Back Pain	11.7	1.7	10.2	0.9
Infection	11.7	2.1	6.4	0.9
Headache	10.5	0.8	14.9	0
Digestive				
Nausea	46.0	5.4	63.0	8.1
Stomatitis	41.4	8.3	15.3	0.4
Vomiting	32.6	7.9	43.8	9.8
Diarrhea	20.9	2.5	34.9	4.2
Anorexia	20.1	2.5	21.7	1.3
Dyspepsia	12.1	0.8	14.0	0
Nervous				
Dizziness	4.2	0	10.2	0
Respiratory				
Pharyngitis	15.9	0	17.9	0.4
Dyspnea	15.1	4.1	23.4	4.3
Cough increased	9.6	0	11.5	0

Non-Hematologic Adverse Reaction 10% or Greater	DOXIL (%) treated (n = 239)		Topotecan (%) treated (n =235)	
	All grades	Grades 3-4	All grades	Grades 3-4
Skin and Appendages				
Hand-foot syndrome	50.6	23.8	0.9	0
Rash	28.5	4.2	12.3	0.4
Alopecia	19.2	N/A	52.3	N/A

The following additional adverse reactions (not in table) were observed in patients with ovarian cancer with doses administered every four weeks.

Incidence 1% to 10%

Cardiovascular: vasodilation, tachycardia, deep thrombophlebitis, hypotension, cardiac arrest.

Digestive: oral moniliasis, mouth ulceration, esophagitis, dysphagia, rectal bleeding, ileus.

Hemic and Lymphatic: ecchymosis.

Metabolic and Nutritional: dehydration, weight loss, hyperbilirubinemia, hypokalemia, hypercalcemia, hyponatremia.

Nervous: somnolence, dizziness, depression.

Respiratory: rhinitis, pneumonia, sinusitis, epistaxis.

Skin and Appendages: pruritus, skin discoloration, vesiculobullous rash, maculopapular rash, exfoliative dermatitis, herpes zoster, dry skin, herpes simplex, fungal dermatitis, furunculosis, acne.

Special Senses: conjunctivitis, taste perversion, dry eyes.

Urinary: urinary tract infection, hematuria, vaginal moniliasis.

Patients With AIDS-Related Kaposi's Sarcoma

The safety data below is based on the experience reported in 753 patients with AIDS-related Kaposi's sarcoma enrolled in four studies. The median age of the population was 38.7 years (range 24-70 years), which was 99% male, 1% female, 88% Caucasian, 6% Hispanic, 4% Black, and 2% Asian/other/unknown. The majority of patients were treated with 20 mg/m^2 of DOXIL every two to three weeks. The median time on study was 127 days and ranged from 1 to 811 days. The median cumulative dose was 120 mg/m^2 and ranged from

3.3 to 798.6 mg/m^2. Twenty-six patients (3.0%) received cumulative doses of greater than 450 mg/m^2.

Of these 753 patients, 61.2% were considered poor risk for KS tumor burden, 91.5% poor for immune system, and 46.9% for systemic illness; 36.2% were poor risk for all three categories. Patients' median CD4 count was 21.0 cells/mm^3, with 50.8% of patients having less than 50 cells/mm^3. The mean absolute neutrophil count at study entry was approximately 3,000 cells/mm^3.

Patients received a variety of potentially myelotoxic drugs in combination with DOXIL. Of the 693 patients with concomitant medication information, 58.7% were on one or more antiretroviral medications, 34.9% patients were on zidovudine (AZT), 20.8% on didanosine (ddI), 16.5% on zalcitabine (ddC), and 9.5% on stavudine (D4T). A total of 85.1% patients were on PCP prophylaxis, most (54.4%) on sulfamethoxazole/trimethoprim. Eighty-five percent of patients were receiving antifungal medications, primarily fluconazole (75.8%). Seventy-two percent of patients were receiving antivirals, 56.3% acyclovir, 29% ganciclovir, and 16% foscarnet. In addition, 47.8% patients received colony-stimulating factors (sargramostim/filgrastim) sometime during their course of treatment.

Adverse reactions led to discontinuation of treatment in 5% of patients with AIDS related Kaposi's sarcoma. Those that did so included bone marrow suppression, cardiac adverse reactions, infusion-related reactions, toxoplasmosis, HFS, pneumonia, cough/dyspnea, fatigue, optic neuritis, progression of a non-KS tumor, allergy to penicillin, and unspecified reasons.

Table 8: Hematology Data Reported in Patients With AIDS-Related Kaposi's Sarcoma

	Patients With Refractory or Intolerant AIDS-Related Kaposi's Sarcoma (n = 74)		Total Patients With AIDS-Related Kaposi's Sarcoma (n = 720)	
Neutropenia				
< 1000/mm^3	34	(45.9%)	352	(48.9%)
< 500/mm^3	8	(10.8%)	96	(13.3%)
Anemia				
< 10 g/dL	43	(58.1%)	399	(55.4%)
< 8 g/dL	12	(16.2%)	131	(18.2%)
Thrombocytopenia				
< 150,000/mm^3	45	(60.8%)	439	(60.9%)
< 25,000/mm^3	1	(1.4%)	30	(4.2%)

Table 9: Probably and Possibly Drug-Related Non-Hematologic Adverse Reactions Reported in ≥ 5% of Patients With AIDS-Related Kaposi's Sarcoma

Adverse Reactions	Patients With Refractory or Intolerant AIDS-Related Kaposi's Sarcoma (n = 77)		Total Patients With AIDS-Related Kaposi's Sarcoma (n = 705)	
Nausea	14	(18.2%)	119	(16.9%)
Asthenia	5	(6.5%)	70	(9.9%)
Fever	6	(7.8%)	64	(9.1%)
Alopecia	7	(9.1%)	63	(8.9%)
Alkaline Phosphatase Increase	1	(1.3%)	55	(7.8%)
Vomiting	6	(7.8%)	55	(7.8%)
Diarrhea	4	(5.2%)	55	(7.8%)
Stomatitis	4	(5.2%)	48	(6.8%)
Oral Moniliasis	1	(1.3%)	39	(5.5%)

The following additional (not in table) adverse reactions were observed in patients with AIDS-related Kaposi's sarcoma.

Incidence 1% to 5%

Body as a Whole: headache, back pain, infection, allergic reaction, chills.

Cardiovascular: chest pain, hypotension, tachycardia.

Cutaneous: herpes simplex, rash, itching.

Digestive: mouth ulceration, anorexia, dysphagia.

Metabolic and Nutritional: SGPT increase, weight loss, hyperbilirubinemia.

Other: dyspnea, pneumonia, dizziness, somnolence.

Incidence Less Than 1%

Body As A Whole: sepsis, moniliasis, cryptococcosis.

Cardiovascular: thrombophlebitis, cardiomyopathy, palpitation, bundle branch block, congestive heart failure, heart arrest, thrombosis, ventricular arrhythmia.

Digestive: hepatitis.

Metabolic and Nutritional Disorders: dehydration

Respiratory: cough increase, pharyngitis.

Skin and Appendages: maculopapular rash, herpes zoster.

Special Senses: taste perversion, conjunctivitis.

Patients With Multiple Myeloma

The safety data below are from 318 patients treated with DOXIL (30 mg/m^2 as a 1-hr i.v. infusion) administered on day 4 following bortezomib (1.3 mg/m^2 i.v. bolus on days 1, 4 , 8 and 11) every three weeks, in a randomized, open-label, multicenter study. In this study, patients in the DOXIL + bortezomib combination group were treated for a median number of 138 days (range 21-410 days). The population was 28-85 years of age, 58% male, 42% female, 90% Caucasian, 6% Black, and 4% Asian and other. Table 10 lists adverse reactions reported in 10% or more of patients treated with DOXIL in combination with bortezomib for multiple myeloma.

Table 10. Frequency of treatment emergent adverse reactions reported in ≥10% patients treated for multiple myeloma with DOXIL in combination with bortezomib, by Severity, Body System, and MedDRA Terminology.

Adverse Reaction	DOXIL + bortezomib (n=318)			Bortezomib (n=318)		
	Any (%)	Grade 3	Grade 4	Any (%)	Grade 3	Grade 4
Blood and lymphatic system disorders						
Neutropenia	36	22	10	22	11	5
Thrombocytopenia	33	11	13	28	9	8
Anemia	25	7	2	21	8	2
General disorders and administration site conditions						
Fatigue	36	6	1	28	3	0
Pyrexia	31	1	0	22	1	0
Asthenia	22	6	0	18	4	0
Gastrointestinal disorders						
Nausea	48	3	0	40	1	0
Diarrhea	46	7	0	39	5	0
Vomiting	32	4	0	22	1	0
Constipation	31	1	0	31	1	0
Mucositis/Stomatitis	20	2	0	5	<1	0
Abdominal pain	11	1	0	8	1	0
Infections and infestations						
Herpes zoster	11	2	0	9	2	0
Herpes simplex	10	0	0	6	1	0
Investigations						
Weight decreased	12	0	0	4	0	0
Metabolism and Nutritional disorders						
Anorexia	19	2	0	14	<1	0
Nervous system disorders						
Peripheral Neuropathy[1]	42	7	<1	45	10	1
Neuralgia	17	3	0	20	4	1
Paresthesia/dysesthesia	13	<1	0	10	0	0
Respiratory, thoracic and mediastinal disorders						
Cough	18	0	0	12	0	0
Skin and subcutaneous tissue disorders						
Rash[2]	22	1	0	18	1	0
Hand-foot syndrome	19	6	0	<1	0	0

Adverse Reaction	DOXIL + bortezomib (n=318)			Bortezomib (n=318)		
	Any (%)	Grade 3	Grade 4	Any (%)	Grade 3	Grade 4

[1] Peripheral neuropathy includes the following adverse reactions: peripheral sensory neuropathy, neuropathy peripheral, polyneuropathy, peripheral motor neuropathy, and neuropathy NOS.

[2] Rash includes the following adverse reactions: rash, rash erythematous, rash macular, rash maculo-papular, rash pruritic, exfoliative rash, and rash generalized.

6.3 Post Marketing Experience

The following additional adverse reactions have been identified during post approval use of DOXIL. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

Musculoskeletal and Connective Tissue Disorders: rare cases of muscle spasms.

Respiratory, Thoracic and Mediastinal Disorders: rare cases of pulmonary embolism (in some cases fatal).

Hematologic disorders: Secondary acute myelogenous leukemia with and without fatal outcome has been reported in patients whose treatment included DOXIL.

Skin and subcutaneous tissue disorders: rare cases of erythema multiforme, Stevens-Johnson syndrome and toxic epidermal necrolysis have been reported.

Secondary oral neoplasms: [see Warnings and Precautions (5.9)].

7 DRUG INTERACTIONS

No formal drug interaction studies have been conducted with DOXIL. DOXIL may interact with drugs known to interact with the conventional formulation of doxorubicin HCl.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category D *[see Warnings and Precautions (5.6)]*.

DOXIL is embryotoxic at doses of 1 mg/kg/day in rats and is embryotoxic and abortifacient at 0.5 mg/kg/day in rabbits (both doses are about one-eighth the 50 mg/m^2 human dose on a mg/m^2 basis). Embryotoxicity was characterized by increased embryo-fetal deaths and reduced live litter sizes.

8.3 Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs, including anthracyclines, are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from DOXIL, discontinue nursing during treatment with DOXIL.

8.4 Pediatric Use

The safety and effectiveness of DOXIL in pediatric patients have not been established.

8.5 Geriatric Use

Of the patients treated with DOXIL in the randomized ovarian cancer study, 34.7% (n=83) were 65 years of age or older while 7.9% (n=19) were 75 years of age or older. Of the 318 patients treated with DOXIL in combination with bortezomib for multiple myeloma, 37% were 65 years of age or older and 8% were 75 years of age or older. No overall differences in safety or efficacy were observed between these patients and younger patients.

8.6 Hepatic Impairment

The pharmacokinetics of DOXIL has not been adequately evaluated in patients with hepatic impairment. Doxorubicin is eliminated in large part by the liver. Thus, DOXIL dosage should be reduced in patients with impaired hepatic function *[see Dosage and Administration (2.6)]*.

Prior to DOXIL administration, evaluation of hepatic function is recommended using conventional clinical laboratory tests such as SGOT, SGPT, alkaline phosphatase, and bilirubin *[see Dosage and Administration (2.6)]*.

10 OVERDOSAGE

Acute overdosage with doxorubicin HCl causes increases in mucositis, leucopenia, and thrombocytopenia.

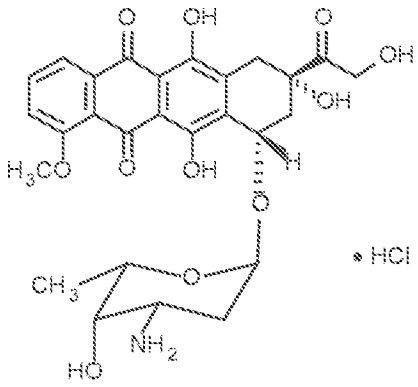
Treatment of acute overdosage consists of treatment of the severely myelosuppressed patient with hospitalization, antibiotics, platelet and granulocyte transfusions, and symptomatic treatment of mucositis.

11 DESCRIPTION

DOXIL (doxorubicin HCl liposome injection) is doxorubicin hydrochloride (HCl) encapsulated in STEALTH® liposomes for intravenous administration.

Doxorubicin is an anthracycline topoisomerase inhibitor isolated from *Streptomyces peucetius* var. *caesius*.

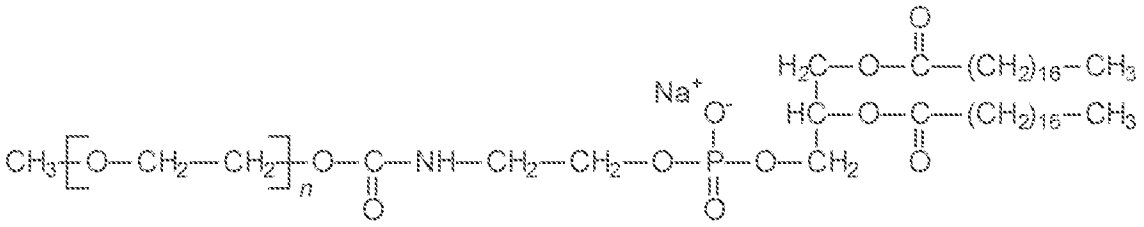
Doxorubicin HCl, which is the established name for (8S,10S)-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-8-glycolyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride, has the following structure:



The molecular formula of the drug is C27 H29 NO11·HCl; its molecular weight is 579.99.

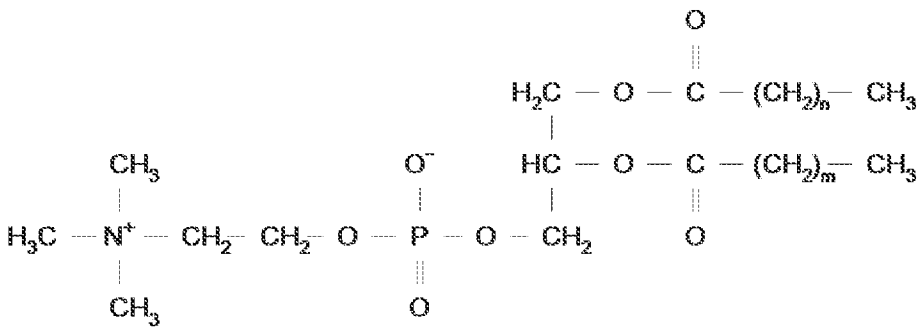
DOXIL is provided as a sterile, translucent, red liposomal dispersion in 10-mL or 30-mL glass, single use vials. Each vial contains 20 mg or 50 mg doxorubicin HCl at a concentration of 2 mg/mL and a pH of 6.5. The STEALTH® liposome carriers are composed of cholesterol, 3.19 mg/mL; fully hydrogenated soy phosphatidylcholine (HSPC), 9.58 mg/mL; and N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (MPEG-DSPE), 3.19 mg/mL. Each mL also contains ammonium sulfate, approximately 2 mg; histidine as a buffer; hydrochloric acid and/or sodium hydroxide for pH control, and sucrose to maintain isotonicity. Greater than 90% of the drug is encapsulated in the STEALTH® liposomes.

MPEG-DSPE has the following structural formula:



n = ca. 45

HSPC has the following structural formula:



m, n = 14 or 16

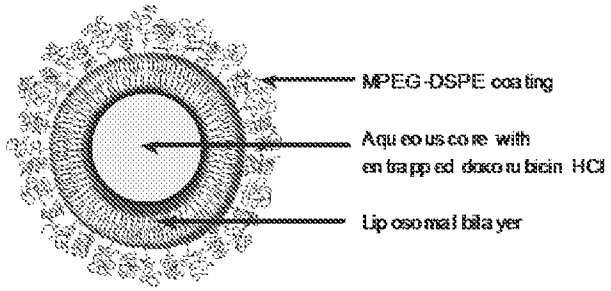
12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

The active ingredient of DOXIL is doxorubicin HCl. The mechanism of action of doxorubicin HCl is thought to be related to its ability to bind DNA and inhibit nucleic acid synthesis. Cell structure studies have demonstrated rapid cell penetration and perinuclear chromatin binding, rapid inhibition of mitotic activity and nucleic acid synthesis, and induction of mutagenesis and chromosomal aberrations.

DOXIL is doxorubicin HCl encapsulated in long-circulating STEALTH® liposomes. Liposomes are microscopic vesicles composed of a phospholipid bilayer that are capable of encapsulating active drugs. The STEALTH® liposomes of DOXIL are formulated with surface-bound methoxypolyethylene glycol (MPEG), a process often referred to as pegylation, to protect liposomes from detection by the mononuclear phagocyte system (MPS) and to increase blood circulation time.

Representation of a STEALTH® liposome:



STEALTH® liposomes have a half-life of approximately 55 hours in humans. They are stable in blood, and direct measurement of liposomal doxorubicin shows that at least 90% of the drug (the assay used cannot quantify less than 5-10% free doxorubicin) remains liposome-encapsulated during circulation.

It is hypothesized that because of their small size (ca. 100 nm) and persistence in the circulation, the pegylated DOXIL liposomes are able to penetrate the altered and often compromised vasculature of tumors. This hypothesis is supported by studies using colloidal gold-containing STEALTH® liposomes, which can be visualized microscopically. Evidence of penetration of STEALTH® liposomes from blood vessels and their entry and accumulation in tumors has been seen in mice with C-26 colon carcinoma tumors and in transgenic mice with Kaposi's sarcoma-like lesions. Once the STEALTH® liposomes distribute to the tissue compartment, the encapsulated doxorubicin HCl becomes available. The exact mechanism of release is not understood.

12.3 Pharmacokinetics

The plasma pharmacokinetics of DOXIL were evaluated in 42 patients with AIDS-related Kaposi's sarcoma (KS) who received single doses of 10 or 20 mg/m^2 administered by a 30-minute infusion. Twenty-three of these patients received single doses of both 10 and 20 mg/m^2 with a 3-week wash-out period between doses. The pharmacokinetic parameter values of DOXIL, given for total doxorubicin (mostly liposomally bound), are presented in Table 11.

Table 11: Pharmacokinetic Parameters of DOXIL in Patients With AIDS-Related Kaposi's Sarcoma

	Dose	
Parameter (units)	10 mg/m^2	20 mg/m^2
Peak Plasma Concentration (μg/mL)	4.12 ± 0.215	8.34 ± 0.49
Plasma Clearance (L/h/m^2)	0.056 ± 0.01	0.041 ± 0.004
Steady State Volume of Distribution (L/m^2)	2.83 ± 0.145	2.72 ± 0.120
AUC (μg/mL•h)	277 ± 32.9	590 ± 58.7
First Phase (λ_1) Half-Life (h)	4.7 ± 1.1	5.2 ± 1.4
Second Phase (λ_1) Half-Life (h)	52.3 ± 5.6	55.0 ± 4.8

N = 23
Mean ± Standard Error

DOXIL displayed linear pharmacokinetics over the range of 10 to 20 mg/m^2. Disposition occurred in two phases after DOXIL administration, with a relatively short first phase (≈ 5 hours) and a prolonged second phase (≈ 55 hours) that accounted for the majority of the area under the curve (AUC).

The pharmacokinetics of DOXIL at a 50 mg/m^2 dose is reported to be nonlinear. At this dose, the elimination half-life of DOXIL is expected to be longer and the clearance lower compared to a 20 mg/m^2 dose. The exposure (AUC) is thus expected to be more than proportional at a 50 mg/m^2 dose when compared with the lower doses.

Distribution:

In contrast to the pharmacokinetics of doxorubicin, which displays a large volume of distribution, ranging from 700 to 1100 L/m^2, the small steady state volume of distribution of DOXIL shows that DOXIL is confined mostly to the vascular fluid volume. Plasma protein binding of DOXIL has not been determined; the plasma protein binding of doxorubicin is approximately 70%.

Metabolism:

Doxorubicinol, the major metabolite of doxorubicin, was detected at very low levels (range: of 0.8 to 26.2 ng/mL) in the plasma of patients who received 10 or 20 mg/m^2 DOXIL.

Excretion:

The plasma clearance of DOXIL was slow, with a mean clearance value of 0.041 L/h/m^2 at a dose of 20 mg/m^2. This is in contrast to doxorubicin, which displays a plasma clearance value ranging from 24 to 35 L/h/m^2.

Because of its slower clearance, the AUC of DOXIL, primarily representing the circulation of liposome-encapsulated doxorubicin, is approximately two to three orders of magnitude larger than the AUC for a similar dose of conventional doxorubicin HCl as reported in the literature.

Special Populations:

The pharmacokinetics of DOXIL have not been separately evaluated in women, in members of different ethnic groups, or in individuals with renal or hepatic insufficiency.

Drug-Drug Interactions:

Drug-drug interactions between DOXIL and other drugs, including antiviral agents, have not been adequately evaluated in patients with ovarian cancer, AIDS-related Kaposi's sarcoma or multiple myeloma.

Tissue Distribution in Patients with Kaposi's Sarcoma:

Kaposi's sarcoma lesions and normal skin biopsies were obtained at 48 and 96 hours post infusion of 20 mg/m^2 DOXIL in 11 patients. The concentration of DOXIL in KS lesions was a median of 19 (range, 3-53) times higher than in normal skin at 48 hours post treatment; however, this was not corrected for likely differences in blood content between KS lesions and normal skin. The corrected ratio may lie between 1 and 22 times. Thus, higher concentrations of DOXIL are delivered to KS lesions than to normal skin.

13 NON-CLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, and Impairment of Fertility

Although no studies have been conducted with DOXIL, doxorubicin HCl and related compounds have been shown to have mutagenic and carcinogenic properties when tested in experimental models.

STEALTH® liposomes without drug were negative when tested in Ames, mouse lymphoma and chromosomal aberration assays *in vitro*, and mammalian micronucleus assay *in vivo*.

The possible adverse effects on fertility in males and females in humans or experimental animals have not been adequately evaluated. However, DOXIL resulted in mild to moderate ovarian and testicular atrophy in mice after a single dose of 36 mg/kg (about twice the 50 mg/m^2 human dose on a mg/m^2 basis). Decreased testicular weights and hypospermia were present in rats after repeat doses ≥ 0.25 mg/kg/day (about one thirtieth the 50 mg/m^2 human dose on a mg/m^2 basis), and diffuse degeneration of the seminiferous tubules and a marked decrease in spermatogenesis were observed in dogs after repeat doses of 1 mg/kg/day (about one half the 50 mg/m^2 human dose on a mg/m^2 basis).

14 CLINICAL STUDIES

14.1 Ovarian Cancer

DOXIL (doxorubicin HCl liposome injection) was studied in three open-label, single-arm, clinical studies of 176 patients with metastatic ovarian cancer. One hundred forty-five (145) of these patients were refractory to both paclitaxel- and platinum-based chemotherapy regimens. Refractory ovarian cancer is defined as disease progression while on treatment, or relapse within 6 months of completing treatment. Patients in these studies received DOXIL at 50 mg/m^2 infused over one hour every 3 or 4 weeks for 3-6 cycles or longer in the absence of dose-limiting toxicity or progression of disease.

The baseline demographics and clinical characteristics of the patients with refractory ovarian cancer are provided in Table 12 below.

Table 12: Patient Demographics for Patients With Refractory Ovarian Cancer From Single Arm Ovarian Cancer Studies

	Study 1 (U.S.) (n = 27)	Study 2 (U.S.) (n = 82)	Study 3 (non-U.S.) (n = 36)
Age at Diagnosis (Years)			
Median	64	61.5	51.5
Range	46 – 75	34 – 85	22 – 80
Drug-Free Interval (Months)			
Median	1.8	1.7	2.6
Range	0.5 – 15.6	0.6 – 7.0	0.7 – 15.2
Sum of Lesions at			

Baseline (cm^2)			
Median	25	18.3	32.4
Range	1.2 – 230.0	1.3 – 285.0	0.3 – 114.0
FIGO Staging			
I	1 (3.7%)	3 (3.7%)	4 (11.1%)
II	3 (11.1%)	3 (3.7%)	1 (2.8%)
III	15 (55.6%)	60 (73.2%)	24 (66.7%)
IV	8 (29.6%)	16 (19.5%)	6 (16.7%)	
Not Specified	----	----	1 (2.8%)
CA-125 at Baseline			
Median	123.5	199.0	1004.5
Range	20 – 14,012	7 – 46,594	20 – 12,089
Number of Prior Chemotherapy Regimens			
1	7 (25.9%)	13 (15.9%)	9 (25.0%)
2	11 (40.7%)	44 (53.7%)	19 (52.8%)
3	6 (22.2%)	25 (30.5%)	8 (22.8%)
4	3 (11.1%)	----	----

The primary efficacy parameter was response rate for the population of patients refractory to both paclitaxel- and a platinum-containing regimen. Assessment of response was based on Southwest Oncology Group (SWOG) criteria, and required confirmation four weeks after the initial observation. Secondary efficacy parameters were time to response, duration of response, and time to progression.

The response rates for the individual single arm studies are given in Table 13 below.

Table 13: Response Rates in Patients With Refractory Ovarian Cancer From Single Arm Ovarian Cancer Studies

	Study 1 (U.S.)	Study 2 (U.S.)	Study 3 (non-U.S.)
Response Rate	22.2% (6/27)	17.1% (14/82)	0% (0/36)
95% Confidence Interval	8.6% - 42.3%	9.7% - 27.0%	0.0% - 9.7%

When the data from the single arm studies are combined, the response rate for all patients refractory to paclitaxel and platinum agents was 13.8% (20/145) (95% CI 8.1% to 19.3%). The median time to progression was 15.9 weeks, the median time to response was 17.6 weeks, and the duration of response was 39.4 weeks.

DOXIL (doxorubicin HCl liposome injection) was also studied in a randomized, multicenter, open-label, study in 474 patients with epithelial ovarian cancer after platinum-based chemotherapy. Patients in this study received an initial dose of either DOXIL 50 mg/m^2 infused over one hour every 4 weeks or topotecan 1.5 mg/m^2 infused daily for 5 consecutive days every 3 weeks. Patients were stratified according to platinum sensitivity and the presence of bulky disease (presence of tumor mass greater than 5 cm in size). Platinum sensitivity is defined by response to initial platinum-based therapy and a

progression-free interval of greater than 6 months off treatment. The primary efficacy endpoint for this study was time to progression (TTP). Other efficacy endpoints included overall survival and objective response rate.

The baseline patient demographic and clinical characteristics are provided in Table 14 below.

Table 14: Ovarian Cancer Randomized Study Baseline Demographic and Clinical Characteristics

	DOXIL (n = 239)	Topotecan (n = 235)
Age at Diagnosis (Years)		
Median	60.0	60.0
Range	27 – 87	25 – 85
Drug-Free Interval (Months)		
Median	7.0	6.7
Range	0.9 – 82.1	0.5 – 109.6
FIGO Staging		
I	11 (4.6%)	15 (6.4%)
II	13 (5.4%)	8 (3.4%)
III	175 (73.2%)	164 (69.8%)
IV	40 (16.7%)	48 (20.4%)
Platinum Sensitivity		
Sensitive	109 (45.6%)	110 (46.8%)
Refractory	130 (54.4%)	125 (53.2%)
Bulky Disease		
Present	108 (45.2%)	105 (44.7%)
Absent	131 (54.8%)	130 (55.3%)

Study results are provided in Table 15.

There was no statistically significant difference in TTP between the two treatment arms.

Table 15: Results of Efficacy Analyses[1]

	Protocol Defined ITT Population	
	DOXIL (n = 239)	Topotecan (n = 235)
TTP (Protocol Specified Primary Endpoint)		
Median (Months)[2]	4.1	4.2
p-value[3]	0.617	
Hazard Ratio[4]	0.955	
95% CI for Hazard Ratio	(0.762, 1.196)	
Overall Survival		
Median (Months) [2]	14.4	13.7
p-value[3]	0.05	
Hazard Ratio[4]	0.822	
95% CI for Hazard Ratio	(0.676, 1.000)	
Response Rate		
Overall Response n (%)	47 (19.7)	40 (17.0)
Complete Response n (%)	9 (3.8)	11 (4.7)

	Protocol Defined ITT Population	
	DOXIL (n = 239)	Topotecan (n = 235)
Partial Response n (%)	38 (15.9)	29 (12.3)
Median Duration of Response (Months)[2]	6.9	5.9

[1] Analysis based on investigators' strata for protocol defined ITT population.
[2] Kaplan-Meier estimates.
[3] p-value is based on the stratified log-rank test.
[4] Hazard ratio is based on Cox proportional-hazard model with the treatment as single independent variable. A hazard ratio less than 1 indicates an advantage for DOXIL.
[5] p-value not adjusted for multiple comparisons.

14.2 AIDS-Related Kaposi's Sarcoma

DOXIL was studied in an open-label, single-arm, multicenter study utilizing DOXIL at 20 mg/m^2 by intravenous infusion every three weeks, generally until progression or intolerance occurred. In an interim analysis, the treatment history of 383 patients was reviewed, and a cohort of 77 patients was retrospectively identified as having disease progression on prior systemic combination chemotherapy (at least 2 cycles of a regimen containing at least two of three treatments: bleomycin, vincristine or vinblastine, or doxorubicin) or as being intolerant to such therapy. Forty-nine of the 77 (64%) patients had received prior doxorubicin HCl.

These 77 patients were predominantly Caucasian, homosexual males with a median CD4 count of 10 cells/mm^3. Their age ranged from 24 to 54 years, with a mean age of 38 years. Using the ACTG staging criteria, 78% of the patients were at poor risk for tumor burden, 96% at poor risk for immune system, and 58% at poor risk for systemic illness at baseline. Their mean Karnofsky status score was 74%. All 77 patients had cutaneous or subcutaneous lesions, 40% also had oral lesions, 26% pulmonary lesions, and 14% of patients had lesions of the stomach/intestine.

The majority of these patients had disease progression on prior systemic combination chemotherapy.

The median time on study for these 77 patients was 155 days and ranged from 1 to 456 days. The median cumulative dose was 154 mg/m^2 and ranged from 20 to 620 mg/m^2.

Two analyses of tumor response were used to evaluate the effectiveness of DOXIL: one analysis based on investigator assessment of changes in lesions over the entire body, and one analysis based on changes in indicator lesions.

Investigator Assessment

Investigator response was based on modified ACTG criteria. Partial response was defined as no new lesions, sites of disease, or worsening edema; flattening of ≥50% of previously raised lesions or area of indicator lesions decreasing by ≥50%; and response lasting at least 21 days with no prior progression.

Indicator Lesion Assessment

A retrospectively defined analysis was conducted based on assessment of the response of up to five prospectively identified representative indicator lesions. A partial response was defined as flattening of ≥50% of previously raised indicator lesions, or >50% decrease in the area of indicator lesions and lasting at least 21 days with no prior progression.

Only patients with adequate documentation of baseline status and follow-up assessments were considered evaluable for response. Patients who received concomitant KS treatment during study, who completed local radiotherapy to sites encompassing one or more of the indicator lesions within two months of study entry, who had less than four indicator lesions, or who had less than three raised indicator lesions at baseline (the latter applies solely to indicator lesion assessment) were considered nonevaluable for response. Of the 77 patients who had disease progression on prior systemic combination chemotherapy or who were intolerant to such therapy, 34 were evaluable for investigator assessment and 42 were evaluable for indicator lesion assessment.

Table 16: Response in Patients with Refractory[1] AIDS-related Kaposi's Sarcoma

Investigator Assessment	All Evaluable Patients (n = 34)	Evaluable Patients Who Received Prior Doxorubicin (n = 20)
Response[2]		
Partial (PR)	27%	30%
Stable	29%	40%
Progression	44%	30%
Duration of PR (Days)		
Median	73	89
Range	42+ - 210+	42+ - 210+
Time to PR (Days)		
Median	43	53
Range	15 – 133	15 – 109
Indicator Lesion Assessment	**All Evaluable Patients (n = 42)**	**Evaluable Patients Who Received Prior Doxorubicin (n = 23)**
Response[2]		
Partial (PR)	48%	52%
Stable	26%	30%
Progression	26%	17%
Duration of PR (Days)		
Median	71	79
Range	22+ - 210+	35 - 210+
Time to PR (Days)		
Median	22	48
Range	15 – 109	15 – 109

[1] Patients with disease that progressed on prior combination chemotherapy or who were intolerant to such therapy.
[2] There were no complete responses in this population.

Retrospective efficacy analyses were performed on two studies that had subsets of patients who received single agent DOXIL and who were on stable antiretroviral therapy for at least 60 days prior to enrollment and at least until a response was demonstrated. In one cooperative group trial that was closed early due to slow accrual, 7 of 17 patients (40%) on stable antiretroviral therapy had a durable response. The median duration was not reached but was longer than 11.6 months. In another trial, 4 of 11 patients (40%) on stable antiretroviral therapy demonstrated durable responses.

14.3 Multiple Myeloma

The safety and efficacy of DOXIL in combination with bortezomib in the treatment of multiple myeloma were evaluated in a randomized, open label, international multicenter study. This study included 646 patients who have not previously received bortezomib and whose disease progressed during or after at least one prior therapy. Patients were

randomized (1:1 ratio) to receive either DOXIL (30 mg/m^2 as a 1-hr i.v. infusion) administered on day 4 following bortezomib (1.3 mg/m^2 i.v. bolus on days 1, 4 , 8 and 11) or bortezomib alone (1.3 mg/m^2 i.v. bolus on days 1, 4 , 8 and 11). Treatment was administered every 3 weeks. Patients were treated for up to 8 cycles until disease progression or the occurrence of unacceptable toxicity. Patients who maintained a response were allowed to receive further treatment. The median number of cycles in each treatment arm was 5 (range 1-18). The baseline demographics and clinical characteristics of the patients with multiple myeloma are provided in Table 17 below.

Table 17 Summary of Baseline Patient and Disease Characteristics

Patient Characteristics	DOXIL + bortezomib n=324	bortezomib n=322
Median age in years (range)	61 (28, 85)	62 (34, 88)
% Male/female	58 / 42	54 / 46
% Caucasian/Black/other	90 / 6/ 4	94 / 4 / 2%
Disease Characteristics		
% with IgG/IgA/Light chain	57 / 27 / 12	62 / 24 /11
% β_2-microglobulin group		
≤2.5 mg/L	14	14
>2.5 mg/L and ≤5.5 mg/L	56	55
>5.5 mg/L	30	31
Serum M-protein (g/dL): Median (Range)	2.5 (0-10.0)	2.7 (0-10.0)
Urine M-protein (mg/24 hours): Median (Range)	107 (0-24883)	66 (0-39657)
Median Months Since Diagnosis	35.2	37.5
% Prior Therapy		
One	34	34
More than one	66	66
Prior Systemic Therapies for Multiple Myeloma		
Corticosteroid (%)	99	>99
Antiracyclines	68	67
Alkylating agent (%)	92	90
Thalidomide/lenalidomide (%)	40	43
Stem cell transplantation (%)	57	54

The primary endpoint in this study was time to progression (TTP). TTP was defined as the time from randomization to the first occurrence of progressive disease or death due to progressive disease. The combination arm demonstrated significant improvement in TTP. As the prespecified primary objective was achieved at the interim analysis, patients in the bortezomib monotherapy group were then allowed to receive the DOXIL + bortezomib combination. Survival continued to be followed after the interim analysis and survival data are not mature at this time. Efficacy results are as shown in Table 18 and Figure 1.

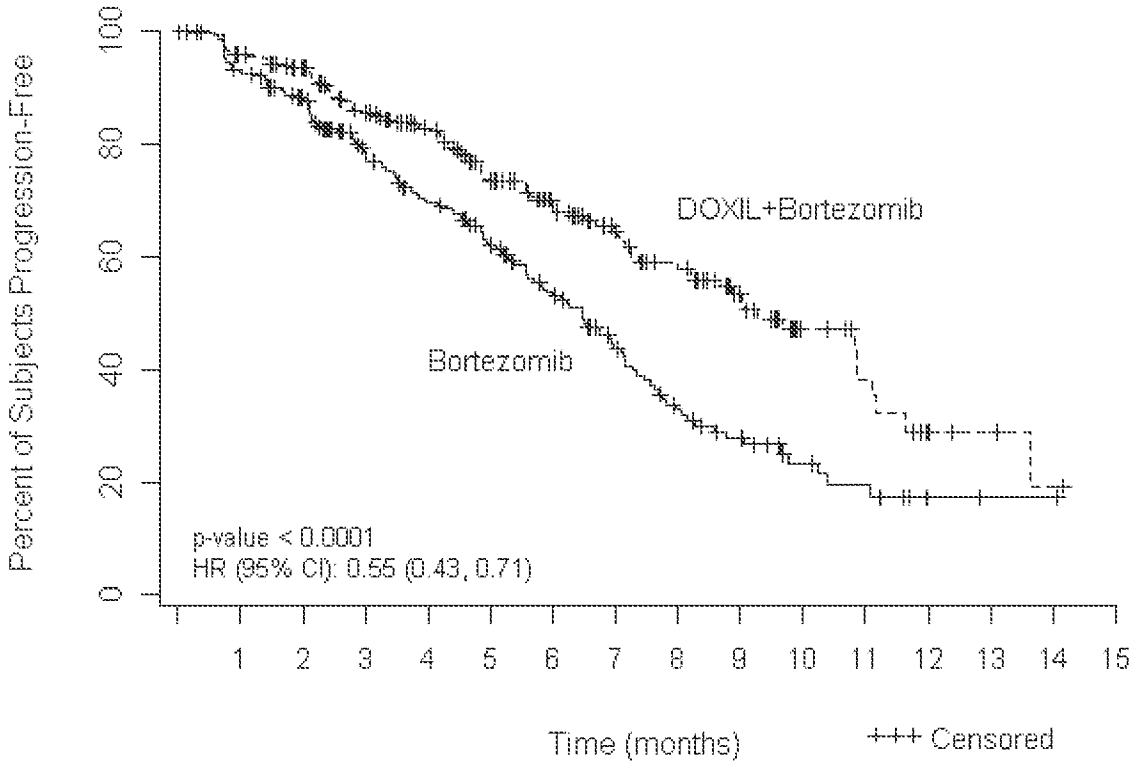
Table 18 Efficacy of DOXIL in combination with bortezomib in the treatment of patients with multiple myeloma

Endpoint	DOXIL + bortezomib n=324		Bortezomib n=322
Time to Progression[1]			
Progression or death due to progression (n)	99		150
Censored (n)	225		172
Median in days (months)	282 (9.3)		197 (6.5)
95% CI	250;338		170;217
Hazard ratio[2]		0.55	
(95% CI)		(0.43, 0.71)	
p-value[3]		<0.0001	
Response (n)[4]	303		310
% Complete Response (CR)	5		3
%Partial Response (PR)	43		40
%CR + PR	48		43
p-value[5]		0.251	
Median Duration of Response (months)	10.2		7.0
(95% CI)	(10.2;12.9)		(5.9;8.3)

[1] Kaplan Meier estimate.
[2] Hazard ratio based on stratified Cox proportional hazards regression. A hazard ratio < 1 indicates an advantage for DOXIL+bortezomib.
[3] Stratified log-rank test.
[4] RR as per EBMT criteria.
[5] Cochran-Mantel-Haenszel test adjusted for the stratification factors.

Time to progression outcomes were consistent with the overall result across most subgroups defined by patient demographic and baseline characteristics. There were too few Blacks or Asian patients to adequately assess differences in effects for the race subgroup.

Figure 1- Time to Progression Kaplan-Meier Curve



Number of Subjects at Risk

DOXIL+Bortezomib	324	301	269	201	170	127	97	70	56	38	19	13	6	4	2	0
Bortezomib	322	290	253	189	150	112	84	56	35	25	14	9	2	1	1	0

15 REFERENCES

1. NIOSH Alert: Preventing occupational exposures to antineoplastic and other hazardous drugs in healthcare settings. 2004. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2004-165.

2. OSHA Technical Manual, TED 1-0.15A, Section VI: Chapter 2. Controlling Occupational Exposure to Hazardous Drugs. OSHA, 1999. http://www.osha.gov/dts/osta/otm/otm_vi/otm_vi_2.html

3. NIH [2002]. 1999 recommendations for the safe handling of cytotoxic drugs. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NIH Publication No. 92-2621.

4. American Society of Health-System Pharmacists. (2006) ASHP Guidelines on Handling Hazardous Drugs.

5. Polovich, M., White, J. M., & Kelleher, L.O. (eds.) 2005. Chemotherapy and biotherapy guidelines and recommendations for practice (2nd. ed.) Pittsburgh, PA: Oncology Nursing Society.

16 HOW SUPPLIED/STORAGE AND HANDLING

DOXIL (doxorubicin HCl liposome injection) is supplied as a sterile, translucent, red liposomal dispersion in 10-mL or 30-mL glass, single use vials.

Each 10-mL vial contains 20 mg doxorubicin HCl at a concentration of 2 mg/mL.

Each 30-mL vial contains 50 mg doxorubicin HCl at a concentration of 2 mg/mL.

Refrigerate unopened vials of DOXIL at 2°-8°C (36°-46°F). Avoid freezing. Prolonged freezing may adversely affect liposomal drug products; however, short-term freezing (less than 1 month) does not appear to have a deleterious effect on DOXIL.

The following individually cartoned vials are available:

Table 19

mg in vial	fill volume	vial size	NDC #s
20 mg vial	10-mL	10-mL	59676-960-01
50 mg vial	25-mL	30-mL	59676-960-02

17 PATIENT COUNSELING INFORMATION

Patients and patients' caregivers should be informed of the expected adverse effects of DOXIL, particularly hand-foot syndrome, stomatitis, and neutropenia and related complications of neutropenic fever, infection, and sepsis.

Hand-Foot Syndrome (HFS): Patients who experience tingling or burning, redness, flaking, bothersome swelling, small blisters, or small sores on the palms of their hands or soles of their feet (symptoms of Hand-Foot Syndrome) should notify their physician.

Stomatitis: Patients who experience painful redness, swelling, or sores in the mouth (symptoms of stomatitis) should notify their physician.

Fever and Neutropenia: Patients who develop a fever of 100.5°F or higher should notify their physician.

Nausea, vomiting, tiredness, weakness, rash, or mild hair loss: Patients who develop any of these symptoms should notify their physician.

Following its administration, DOXIL may impart a reddish-orange color to the urine and other body fluids. This nontoxic reaction is due to the color of the product and will dissipate as the drug is eliminated from the body.

Manufactured by:
Ben Venue Laboratories, Inc.
Bedford, OH 44146

Manufactured for:
Janssen Products, LP
Horsham, PA 19044

Revised August 2013

An ALZA STEALTH®

Technology Product

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use DOXIL safely and effectively. See full prescribing information for DOXIL.

DOXIL® (doxorubicin HCl liposome injection) for intravenous infusion
Initial U.S. Approval: 1995

WARNING: INFUSION REACTIONS, MYELOSUPPRESSION, CARDIOTOXICITY, LIVER IMPAIRMENT, SUBSTITUTION

See full prescribing information for complete boxed warning.

- **Myocardial damage may lead to congestive heart failure and may occur as the total cumulative dose of doxorubicin HCl approaches 550 mg/m^2. Cardiac toxicity may also occur at lower cumulative doses with mediastinal irradiation or concurrent cardiotoxic agents (5.1).**
- **Acute infusion-related reactions, sometimes reversible upon terminating or slowing infusion, occurred in up to 10% of patients. Serious and sometimes fatal allergic/anaphylactoid-like infusion reactions have been reported. Medications/emergency equipment to treat such reactions should be available for immediate use (5.2).**
- **Severe myelosuppression may occur (5.3)**
- **Reduce dosage in patients with impaired hepatic function (2.6).**
- **Accidental substitution of DOXIL resulted in severe side effects. Do not substitute on mg per mg basis with doxorubicin HCl (2.1).**

----------RECENT MAJOR CHANGES----------

Indications and Usage, AIDS-related Kaposi's Sarcoma (1.2) 6/2008

----------INDICATIONS AND USAGE----------

DOXIL is an anthracycline topoisomerase inhibitor indicated for:

- **Ovarian cancer (1.1)**

After failure of platinum-based chemotherapy.

- **AIDS-related Kaposi's Sarcoma (1.2)**

After failure of prior systemic chemotherapy or intolerance to such therapy.

- **Multiple Myeloma (1.3)**

In combination with bortezomib in patients who have not previously received bortezomib and have received at least one prior therapy.

----------DOSAGE AND ADMINISTRATION----------

Administer DOXIL at an initial rate of 1 mg/min to minimize the risk of infusion reactions. If no infusion related reactions occur, increase rate of infusion to complete administration over 1 hour. Do not administer as bolus injection or undiluted solution (2.1).

- **Ovarian cancer:** 50 mg/m^2 IV every 4 weeks for 4 courses minimum (2.2)
- **AIDS-related Kaposi's Sarcoma:** 20 mg/m^2 IV every 3 weeks (2.3)
- **Multiple Myeloma:** 30 mg/m^2 IV on day 4 following bortezomib which is administered at 1.3 mg/m^2 bolus on days 1, 4, 8 and 11, every 3 weeks (2.4)

----------DOSAGE FORMS AND STRENGTHS----------

Single dose vial: 20 mg/10 mL and 50 mg/30 mL (3)

----------CONTRAINDICATIONS----------

- Hypersensitivity reactions to a conventional formulation of doxorubicin HCl or the components of DOXIL (4, 5.2)
- Nursing mothers (4, 8.3)

----------WARNINGS AND PRECAUTIONS----------

- Hand-Foot Syndrome may occur. Dose modification or discontinuation may be required (5.4)
- Radiation recall reaction may occur (5.5)

----------ADVERSE REACTIONS----------

Most common adverse reactions (>20%) are asthenia, fatigue, fever, anorexia, nausea, vomiting, stomatitis, diarrhea, constipation, hand and foot syndrome, rash, neutropenia, thrombocytopenia and anemia (6).

To report SUSPECTED ADVERSE REACTIONS contact Ortho Biotech Products, LP at (888) 227-5624 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

----------DRUG INTERACTIONS----------

- DOXIL may interact with drugs known to interact with conventional formulations of Doxorubicin HCl. (7)

----------USE IN SPECIFIC POPULATIONS----------

- DOXIL can cause fetal harm when used during pregnancy. (5.6, 8.1)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 06/2008

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FULL PRESCRIBING INFORMATION

WARNING: INFUSION REACTIONS, MYELOSUPPRESSION, CARDIOTOXICITY, LIVER IMPAIRMENT, ACCIDENTAL SUBSTITUTION

1. **The use of DOXIL (doxorubicin HCl liposome injection) may lead to cardiac toxicity. Myocardial damage may lead to congestive heart failure and may occur as the total cumulative dose of doxorubicin HCl approaches 550 mg/m^2. In a clinical study in patients with advanced breast cancer, 250 patients received DOXIL at a starting dose of 50 mg/m^2 every 4 weeks. At all cumulative anthracycline doses between 450-500 mg/m^2 or between 500-550 mg/m^2, the risk of cardiac toxicity for patients treated with DOXIL was 11%. Prior use of other anthracyclines or anthracenediones should be included in calculations of total cumulative dosage. Cardiac toxicity may also occur at lower cumulative doses in patients with prior mediastinal irradiation or who are receiving concurrent cyclophosphamide therapy *[see Warnings and Precautions (5.1)]*.**

2. **Acute infusion-related reactions including, but not limited to, flushing, shortness of breath, facial swelling, headache, chills, back pain, tightness in the chest or throat, and/or hypotension have occurred in up to 10% of patients treated with DOXIL. In most patients, these reactions resolve over the course of several hours to a day once the infusion is terminated. In some patients, the reaction has resolved with slowing of the infusion rate. Serious and sometimes life-threatening or fatal allergic/anaphylactoid-like infusion reactions have been reported. Medications to treat such reactions, as well as emergency equipment, should be available for immediate use. DOXIL should be administered at an initial rate of 1 mg/min to minimize the risk of infusion reactions *[see Warnings and Precautions (5.2)]*.**

3. **Severe myelosuppression may occur *[see Warnings and Precautions (5.3)]*.**

4. **Dosage should be reduced in patients with impaired hepatic function *[see Dosage and Administration (2.6) and Use in Specific Populations (8.6)]*.**

5. **Accidental substitution of DOXIL for doxorubicin HCl has resulted in severe side effects. DOXIL should not be substituted for doxorubicin HCl on a mg per mg basis *[see Dosage and Administration (2.1)]*.**

1 INDICATIONS AND USAGE

1.1 Ovarian Cancer

DOXIL (doxorubicin HCl liposome injection) is indicated for the treatment of patients with ovarian cancer whose disease has progressed or recurred after platinum-based chemotherapy.

1.2 AIDS-Related Kaposi's Sarcoma

DOXIL is indicated for the treatment of AIDS-related Kaposi's sarcoma in patients after failure of prior systemic chemotherapy or intolerance to such therapy.

1.3 Multiple Myeloma

DOXIL in combination with bortezomib is indicated for the treatment of patients with multiple myeloma who have not previously received bortezomib and have received at least one prior therapy.

2 DOSAGE AND ADMINISTRATION

2.1 Usage and Administration Precautions

Liposomal encapsulation can substantially affect a drug's functional properties relative to those of the unencapsulated drug. Therefore DO NOT SUBSTITUTE one drug for the other.

Do not administer as a bolus injection or an undiluted solution. Rapid infusion may increase the risk of infusion-related reactions *[see Warnings and Precautions (5.2)]*. DOXIL must not be given by the intramuscular or subcutaneous route.

Until specific compatibility data are available, it is not recommended that DOXIL be mixed with other drugs.

DOXIL should be considered an irritant and precautions should be taken to avoid extravasation. With intravenous administration of DOXIL, extravasation may occur with or without an accompanying stinging or burning sensation, even if blood returns well on aspiration of the infusion needle. If any signs or symptoms of extravasation have occurred, the infusion should be immediately terminated and restarted in another vein. The application of ice over the site of extravasation for approximately 30 minutes may be helpful in alleviating the local reaction.

2.2 Patients With Ovarian Cancer

DOXIL (doxorubicin HCl liposome injection) should be administered intravenously at a dose of 50 mg/m^2 (doxorubicin HCl equivalent) at an initial rate of 1 mg/min to minimize the risk of infusion reactions. If no infusion-related adverse reactions are observed, the rate of infusion can be increased to complete administration of the drug over one hour. The patient should be dosed once every 4 weeks, for as long as the patient does not progress, shows no evidence of cardiotoxicity *[see Warnings and Precautions (5.1)]*, and continues to tolerate treatment. A minimum of 4 courses is recommended because median time to response in clinical trials was 4 months. To manage adverse reactions such as hand-foot syndrome (HFS), stomatitis, or hematologic toxicity the doses may be delayed or reduced *[see Dosage and Administration (2.5)]*. Pretreatment with or concomitant use of antiemetics should be considered.

2.3 Patients With AIDS-Related Kaposi's Sarcoma

DOXIL (doxorubicin HCl liposome injection) should be administered intravenously at a dose of 20 mg/m^2 (doxorubicin HCl equivalent). An initial rate of 1 mg/min should be used to

minimize the risk of infusion-related reactions. If no infusion-related adverse reactions are observed, the infusion rate should be increased to complete the administration of the drug over one hour. The dose should be repeated once every three weeks, for as long as patients respond satisfactorily and tolerate treatment.

2.4 Patients With Multiple Myeloma

Bortezomib is administered at a dose of 1.3 mg/m^2 as intravenous bolus on days 1, 4 , 8 and 11, every three weeks. DOXIL 30 mg/m^2 should be administered as a 1-hr intravenous infusion on day 4 following bortezomib. With the first DOXIL dose, an initial rate of 1 mg/min should be used to minimize the risk of infusion-related reactions. If no infusion-related adverse reactions are observed, the infusion rate should be increased to complete the administration of the drug over one hour. Patients may be treated for up to 8 cycles until disease progression or the occurrence of unacceptable toxicity.

2.5 Dose Modification Guidelines

DOXIL exhibits nonlinear pharmacokinetics at 50 mg/m^2; therefore, dose adjustments may result in a non-proportional greater change in plasma concentration and exposure to the drug *[see Clinical Pharmacology (12.3)]*.

Patients should be carefully monitored for toxicity. Adverse reactions, such as HFS, hematologic toxicities, and stomatitis may be managed by dose delays and adjustments. Following the first appearance of a Grade 2 or higher adverse reactions, the dosing should be adjusted or delayed as described in the following tables. Once the dose has been reduced, it should not be increased at a later time.

Recommended Dose Modification Guidelines

Table 1: Hand-Foot Syndrome (HFS)

Toxicity Grade	Dose Adjustment
1 (mild erythema, swelling, or desquamation not interfering with daily activities)	**Redose unless patient has experienced previous Grade 3 or 4 HFS.** If so, delay up to 2 weeks and decrease dose by 25%. Return to original dose interval.
2 (erythema, desquamation, or swelling interfering with, but not precluding normal physical activities; small blisters or ulcerations less than 2 cm in diameter)	**Delay dosing up to 2 weeks or until resolved to Grade 0-1.** If after 2 weeks there is no resolution, DOXIL should be discontinued. If resolved to Grade 0-1 within 2 weeks, and there are no prior Grade 3-4 HFS, continue treatment at previous dose and return to original dose interval. If patient experienced previous Grade 3-4 toxicity, continue treatment with a 25% dose reduction and return to original dose interval.
3 (blistering, ulceration, or swelling interfering with walking or normal daily activities; cannot wear regular clothing)	**Delay dosing up to 2 weeks or until resolved to Grade 0-1.** Decrease dose by 25% and return to original dose interval. If after 2 weeks there is no resolution, DOXIL should be discontinued.
4 (diffuse or local process causing infectious complications, or a bed ridden state or hospitalization)	**Delay dosing up to 2 weeks or until resolved to Grade 0-1.** Decrease dose by 25% and return to original dose interval. If after 2 weeks there is no resolution, DOXIL should be discontinued.

Table 2: Hematological Toxicity

Grade	ANC	Platelets	Modification
1	1,500 – 1,900	75,000 - 150,000	Resume treatment with no dose reduction
2	1,000 - <1,500	50,000 - <75,000	Wait until ANC $\geq$ 1,500 and platelets $\geq$ 75,000; redose with no dose reduction
3	500 – 999	25,000 - <50,000	Wait until ANC $\geq$ 1,500 and platelets $\geq$ 75,000; redose with no dose reduction
4	<500	<25,000	Wait until ANC $\geq$ 1,500 and platelets $\geq$ 75,000; redose at 25% dose reduction or continue full dose with cytokine support

Table 3: Stomatitis

Toxicity Grade	Dose Adjustment
1 (painless ulcers, erythema, or mild soreness)	**Redose unless patient has experienced previous Grade 3 or 4 toxicity.** If so, delay up to 2 weeks and decrease dose by 25%. Return to original dose interval.
2 (painful erythema, edema, or ulcers, but can eat)	**Delay dosing up to 2 weeks or until resolved to Grade 0-1.** If after 2 weeks there is no resolution, DOXIL should be discontinued. If resolved to Grade 0-1 within 2 weeks and there was no prior Grade 3-4 stomatitis, continue treatment at previous dose and return to original dose interval. If patient experienced previous Grade 3-4 toxicity, continue treatment with a 25% dose reduction and return to original dose interval.
3 (painful erythema, edema, or ulcers, and cannot eat)	**Delay dosing up to 2 weeks or until resolved to Grade 0-1.** Decrease dose by 25% and return to original dose interval. If after 2 weeks there is no resolution, DOXIL should be discontinued.
4 (requires parenteral or enteral support)	**Delay dosing up to 2 weeks or until resolved to Grade 0-1.** Decrease dose by 25% and return to DOXIL original dose interval. If after 2 weeks there is no resolution, DOXIL should be discontinued.

Multiple Myeloma

For patients treated with DOXIL in combination with bortezomib who experience hand-foot syndrome or stomatitis, the DOXIL dose should be modified as described in Tables 1 and 3 above. Table 4 describes dosage adjustments for DOXIL and bortezomib combination therapy. For bortezomib dosing and dosage adjustments, see manufacturer's prescribing information.

Table 4: Dosage adjustments for DOXIL + bortezomib combination therapy

Patient status	DOXIL	bortezomib
Fever ≥38°C and ANC <1,000/mm^3	Do not dose this cycle if before Day 4; if after Day 4, reduce next dose by 25%.	Reduce next dose by 25%
On any day of drug administration after Day 1 of each cycle: Platelet count <25,000/mm^3 Hemoglobin <8g/dL ANC <500/mm^3	Do not dose this cycle if before Day 4; if after Day 4 reduce next dose by 25% in the following cycles if bortezomib is reduced for hematologic toxicity.	Do not dose; if 2 or more doses are not given in a cycle, reduce dose by 25% in following cycles.
Grade 3 or 4 non-hematologic drug related toxicity	Do not dose until recovered to Grade <2 and reduce dose by 25% for all subsequent doses.	Do not dose until recovered to Grade <2 and reduce dose by 25% for all subsequent doses.
Neuropathic pain or peripheral neuropathy	No dosage adjustments.	See bortezomib manufacturer's prescribing information for dosage adjustments in patients with neuropathic pain.

2.6 Patients With Impaired Hepatic Function

Limited clinical experience exists in treating patients with hepatic impairment with DOXIL. Based on experience with doxorubicin HCl, it is recommended that the DOXIL dosage be reduced if the bilirubin is elevated as follows: serum bilirubin 1.2 to 3.0 mg/dL - give ½ normal dose; serum bilirubin > 3 mg/dL - give ¼ normal dose.

No information, including dosage adjustments, is available for patients with multiple myeloma with hepatic impairment.

2.7 Preparation for Intravenous Administration

Each 10-mL vial contains 20 mg doxorubicin HCl at a concentration of 2 mg/mL.

Each 30-mL vial contains 50 mg doxorubicin HCl at a concentration of 2 mg/mL.

DOXIL doses up to 90 mg must be diluted in 250 mL of 5% Dextrose Injection, USP prior to administration. Doses exceeding 90 mg should be diluted in 500 mL of 5% Dextrose Injection, USP prior to administration. Aseptic technique must be strictly observed since no preservative or bacteriostatic agent is present in DOXIL. Diluted DOXIL should be refrigerated at 2°C to 8°C (36°F to 46°F) and administered within 24 hours.

Do not use with in-line filters.

Do not mix with other drugs.

Do not use with any diluent other than 5% Dextrose Injection.

Do not use any bacteriostatic agent, such as benzyl alcohol.

DOXIL is not a clear solution but a translucent, red liposomal dispersion.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use if a precipitate or foreign matter is present.

Rapid flushing of the infusion line should be avoided.

2.8 Procedure for Proper Handling and Disposal

Caution should be exercised in the handling and preparation of DOXIL.

The use of gloves is required.

If DOXIL comes into contact with skin or mucosa, immediately wash thoroughly with soap and water.

DOXIL should be considered an irritant and precautions should be taken to avoid extravasation. With intravenous administration of DOXIL, extravasation may occur with or without an accompanying stinging or burning sensation, even if blood returns well on aspiration of the infusion needle. If any signs or symptoms of extravasation have occurred, the infusion should be immediately terminated and restarted in another vein. **DOXIL must not be given by the intramuscular or subcutaneous route.**

DOXIL should be handled and disposed of in a manner consistent with other anticancer drugs. Several guidelines on this subject exist *[see References (15)]*.

3 DOSAGE FORMS AND STRENGTHS

- 20 mg/10 mL single use vial
- 50 mg/30 mL single use vial

4 CONTRAINDICATIONS

DOXIL (doxorubicin HCl liposome injection) is contraindicated in patients who have a history of hypersensitivity reactions to a conventional formulation of doxorubicin HCl or the components of DOXIL *[see Warnings and Precautions (5.2)]*.

DOXIL is contraindicated in nursing mothers *[see Use in Specific Populations (8.3)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Cardiac Toxicity

Special attention must be given to the risk of myocardial damage from cumulative doses of doxorubicin HCl. Acute left ventricular failure may occur with doxorubicin, particularly in patients who have received a total cumulative dosage of doxorubicin exceeding the currently recommended limit of 550 mg/m^2. Lower (400 mg/m^2) doses appear to cause heart failure in patients who have received radiotherapy to the mediastinal area or concomitant therapy with other potentially cardiotoxic agents such as cyclophosphamide.

Prior use of other anthracyclines or anthracenodiones should be included in calculations of total cumulative dosage. Congestive heart failure or cardiomyopathy may be encountered after discontinuation of anthracycline therapy. Patients with a history of cardiovascular disease should be administered DOXIL only when the potential benefit of treatment outweighs the risk.

Cardiac function should be carefully monitored in patients treated with DOXIL. The most definitive test for anthracycline myocardial injury is endomyocardial biopsy. Other methods, such as echocardiography or multigated radionuclide scans, have been used to monitor cardiac function during anthracycline therapy. Any of these methods should be employed to monitor potential cardiac toxicity in patients treated with DOXIL. If these test results indicate possible cardiac injury associated with DOXIL therapy, the benefit of continued therapy must be carefully weighed against the risk of myocardial injury.

In a clinical study in patients with advanced breast cancer, 250 patients received DOXIL at starting dose of 50 mg/m^2 every 4 weeks. At all cumulative anthracycline doses between 450-500 mg/m^2, or between 500–550 mg/m^2, the risk of cardiac toxicity for patients treated with DOXIL was 11%. In this study, cardiotoxicity was defined as a decrease of >20% from baseline if the resting left ventricular ejection fraction (LVEF) remained in the normal range, or a decrease of >10% if the resting LVEF became abnormal (less than the institutional lower limit of normal). The data on left ventricular ejection fraction (LVEF) defined cardiotoxicity and congestive heart failure (CHF) are in the table below.

Table 5: Number of Patients With Advanced Breast Cancer

	DOXIL (n=250)
Patients who Developed Cardiotoxicity (LVEF Defined)	10
Cardiotoxicity (With Signs & Symptoms of CHF)	0
Cardiotoxicity (no Signs & Symptoms of CHF)	10
Patients With Signs and Symptoms of CHF Only	2

In the randomized multiple myeloma study, the incidence of heart failure events (ventricular dysfunction, cardiac failure, right ventricular failure, congestive cardiac failure, chronic cardiac

failure, acute pulmonary edema and pulmonary edema) was similar in the DOXIL+bortezomib group and the bortezomib monotherapy group, 3% in each group. LVEF decrease was defined as an absolute decrease of ≥15% over baseline or a ≥5% decrease below the institutional lower limit of normal. Based on this definition, 25 patients in the bortezomib arm (8%) and 42 patients in the DOXIL + bortezomib arm (13%) experienced a reduction in LVEF.

5.2 Infusion Reactions

Acute infusion-related reactions were reported in 7.1% of patients treated with DOXIL in the randomized ovarian cancer study. These reactions were characterized by one or more of the following symptoms: flushing, shortness of breath, facial swelling, headache, chills, chest pain, back pain, tightness in the chest and throat, fever, tachycardia, pruritus, rash, cyanosis, syncope, bronchospasm, asthma, apnea, and hypotension. In most patients, these reactions resolve over the course of several hours to a day once the infusion is terminated. In some patients, the reaction resolved when the rate of infusion was slowed. In this study, two patients treated with DOXIL (0.8%) discontinued due to infusion-related reactions. In clinical studies, six patients with AIDS-related Kaposi's sarcoma (0.9%) and 13 (1.7%) solid tumor patients discontinued DOXIL therapy because of infusion-related reactions.

Serious and sometimes life-threatening or fatal allergic/anaphylactoid-like infusion reactions have been reported. Medications to treat such reactions, as well as emergency equipment, should be available for immediate use.

The majority of infusion-related events occurred during the first infusion. Similar reactions have not been reported with conventional doxorubicin and they presumably represent a reaction to the DOXIL liposomes or one of its surface components.

The initial rate of infusion should be 1 mg/min to help minimize the risk of infusion reactions *[see Dosage and Administration (2)]*.

5.3 Myelosuppression

Because of the potential for bone marrow suppression, careful hematologic monitoring is required during use of DOXIL, including white blood cell, neutrophil, platelet counts, and Hgb/Hct. With the recommended dosage schedule, leukopenia is usually transient. Hematologic toxicity may require dose reduction or delay or suspension of DOXIL therapy. Persistent severe myelosuppression may result in superinfection, neutropenic fever, or hemorrhage. Development of sepsis in the setting of neutropenia has resulted in discontinuation of treatment and, in rare cases, death.

DOXIL may potentiate the toxicity of other anticancer therapies. In particular, hematologic toxicity may be more severe when DOXIL is administered in combination with other agents that cause bone marrow suppression.

In patients with relapsed ovarian cancer, myelosuppression was generally moderate and reversible. In the three single-arm studies, anemia was the most common hematologic adverse reaction (52.6%), followed by leukopenia (WBC< 4,000 mm^3; 42.2%), thrombocytopenia (24.2%), and neutropenia (ANC <1,000; 19.0%). In the randomized study, anemia was the most common hematologic adverse reaction (40.2%), followed by leukopenia (WBC <4,000 mm^3; 36.8%), neutropenia (ANC <1,000; 35.1%), and thrombocytopenia (13.0%) *[see Adverse Reactions (6.2)]*.

In patients with relapsed ovarian cancer, 4.6% received G-CSF (or GM-CSF) to support their blood counts *[see Dosage and Administrations (2.5)]*.

For patients with AIDS-related Kaposi's sarcoma who often present with baseline myelosuppression due to such factors as their HIV disease or concomitant medications, myelosuppression appears to be the dose-limiting adverse reaction at the recommended dose of 20 mg/m^2 *[see Adverse Reactions (6.2)]*. Leukopenia is the most common adverse reaction experienced in this population; anemia and thrombocytopenia can also be expected. Sepsis occurred in 5% of patients; for 0.7% of patients the event was considered possibly or probably related to DOXIL. Eleven patients (1.6%) discontinued study because of bone marrow suppression or neutropenia.

Table 10 presents data on myelosuppression in patients with multiple myeloma receiving DOXIL and bortezomib in combination *[see Adverse Reactions (6.2)]*.

5.4 Hand-Foot Syndrome (HFS)

In the randomized ovarian cancer study, 50.6% of patients treated with DOXIL at 50 mg/m^2 every 4 weeks experienced HFS (developed palmar-plantar skin eruptions characterized by swelling, pain, erythema and, for some patients, desquamation of the skin on the hands and the feet), with 23.8% of the patients reporting HFS Grade 3 or 4 events. Ten subjects (4.2%) discontinued treatment due to HFS or other skin toxicity. HFS toxicity grades are described above *[see definitions of HFS grades in Dosage and Administration (2.5)]*.

Among 705 patients with AIDS-related Kaposi's sarcoma treated with DOXIL at 20 mg/m^2 every 2 weeks, 24 (3.4%) developed HFS, with 3 (0.9%) discontinuing.

In the randomized multiple myeloma study, 19% of patients treated with DOXIL at 30 mg/m^2 every three weeks experienced HFS.

HFS was generally observed after 2 or 3 cycles of treatment but may occur earlier. In most patients the reaction is mild and resolves in one to two weeks so that prolonged delay of therapy need not occur. However, dose modification may be required to manage HFS *[see Dosage and Administration (2.5)]*. The reaction can be severe and debilitating in some patients and may require discontinuation of treatment.

5.5 Radiation Recall Reaction

Recall reaction has occurred with DOXIL administration after radiotherapy.

5.6 Fetal Mortality

Pregnancy Category D

DOXIL can cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies in pregnant women. If DOXIL is to be used during pregnancy, or if the patient becomes pregnant during therapy, the patient should be apprised of the potential hazard to the fetus. If pregnancy occurs in the first few months following treatment with DOXIL, the prolonged half-life of the drug must be considered. Women of childbearing potential should be advised to avoid pregnancy during treatment with Doxil. *[see Use in Specific Populations (8.1)]*.

5.7 Toxicity Potentiation

The doxorubicin in DOXIL may potentiate the toxicity of other anticancer therapies. Exacerbation of cyclophosphamide-induced hemorrhagic cystitis and enhancement of the hepatotoxicity of 6-mercaptopurine have been reported with the conventional formulation of doxorubicin HCl. Radiation-induced toxicity to the myocardium, mucosae, skin, and liver have been reported to be increased by the administration of doxorubicin HCl.

5.8 Monitoring: Laboratory Tests

Complete blood counts, including platelet counts, should be obtained frequently and at a minimum prior to each dose of DOXIL *[see Warnings and Precautions (5.3)]*.

6 ADVERSE REACTIONS

6.1 Overall Adverse Reactions Profile

The following adverse reactions are discussed in more detail in other sections of the labeling.

- Cardiac Toxicity *[see Warnings and Precautions (5.1)]*
- Infusion reactions *[see Warnings and Precautions (5.2)]*
- Myelosuppression *[see Warnings and Precautions (5.3)]*

- Hand-Foot syndrome *[see Warnings and Precautions (5.4)]*

The most common adverse reactions observed with DOXIL are asthenia, fatigue, fever, nausea, stomatitis, vomiting, diarrhea, constipation, anorexia, hand-foot syndrome, rash and neutropenia, thrombocytopenia and anemia.

The most common serious adverse reactions observed with DOXIL are described in Section 6.2.

The safety data described below reflect exposure to DOXIL in 1310 patients including: 239 patients with ovarian cancer, 753 patients with AIDS-related Kaposi's sarcoma and 318 patients with multiple myeloma *[see Adverse Reactions in Clinical Trials (6.2)]*.

6.2 Adverse Reactions in Clinical Trials

Because clinical trials are conducted under widely varying conditions, the adverse reaction rates observed cannot be directly compared to rates on other clinical trials and may not reflect the rates observed in clinical practice.

The following tables present adverse reactions from clinical trials of DOXIL in ovarian cancer and AIDS-Related Kaposi's sarcoma.

Patients With Ovarian Cancer

The safety data described below are from 239 patients with ovarian cancer treated with DOXIL (doxorubicin HCl liposome injection) at 50 mg/m^2 once every 4 weeks for a minimum of 4 courses in a randomized, multicenter, open-label study. In this study, patients received DOXIL for a median number of 98.0 days (range 1-785 days). The population studied was 27-87 years of age, 91% Caucasian, 6% Black and 3% Hispanic and other.

Table 6 presents the hematologic adverse reactions from the randomized study of DOXIL compared to topotecan.

Table 6: Ovarian Cancer Randomized Study Hematology Data Reported in Patients With Ovarian Cancer

	DOXIL Patients (n = 239)	Topotecan Patients (n = 235)
Neutropenia		
500 - <1000/mm^3	19 (7.9%)	33 (14.0%)
<500/mm^3	10 (4.2%)	146 (62.1%)
Anemia		
6.5 - <8 g/dL	13 (5.4%)	59 (25.1%)
< 6.5 g/dL	1 (0.4%)	10 (4.3%)
Thrombocytopenia		
10,000 - <50,000/mm^3	3 (1.3%)	40 (17.0%)
<10,000/mm^3	0 (0.0%)	40 (17.0%)

Table 7 presents a comparative profile of the non-hematologic adverse reactions from the randomized study of DOXIL compared to topotecan.

Table 7: Ovarian Cancer Randomized Study

Non-Hematologic Adverse Reaction 10% or Greater	DOXIL (%) treated (n = 239)		Topotecan (%) treated (n =235)	
	All grades	Grades 3-4	All grades	Grades 3-4
Body as a Whole				
Asthenia	40.2	7.1	51.5	8.1
Fever	21.3	0.8	30.6	5.5
Mucous Membrane Disorder	14.2	3.8	3.4	0
Back Pain	11.7	1.7	10.2	0.9
Infection	11.7	2.1	6.4	0.9
Headache	10.5	0.8	14.9	0
Digestive				
Nausea	46.0	5.4	63.0	8.1
Stomatitis	41.4	8.3	15.3	0.4
Vomiting	32.6	7.9	43.8	9.8
Diarrhea	20.9	2.5	34.9	4.2
Anorexia	20.1	2.5	21.7	1.3
Dyspepsia	12.1	0.8	14.0	0
Nervous				
Dizziness	4.2	0	10.2	0
Respiratory				
Pharyngitis	15.9	0	17.9	0.4
Dyspnea	15.1	4.1	23.4	4.3
Cough increased	9.6	0	11.5	0
Skin and Appendages				
Hand-foot syndrome	50.6	23.8	0.9	0
Rash	28.5	4.2	12.3	0.4
Alopecia	19.2	N/A	52.3	N/A

The following additional adverse reactions (not in table) were observed in patients with ovarian cancer with doses administered every four weeks.

Incidence 1% to 10%

Cardiovascular: vasodilation, tachycardia, deep thrombophlebitis, hypotension, cardiac arrest.

Digestive: oral moniliasis, mouth ulceration, esophagitis, dysphagia, rectal bleeding, ileus.

Hemic and Lymphatic: ecchymosis.

Metabolic and Nutritional: dehydration, weight loss, hyperbilirubinemia, hypokalemia, hypercalcemia, hyponatremia.

Nervous: somnolence, dizziness, depression.

Respiratory: rhinitis, pneumonia, sinusitis, epistaxis.

Skin and Appendages: pruritus, skin discoloration, vesiculobullous rash, maculopapular rash, exfoliative dermatitis, herpes zoster, dry skin, herpes simplex, fungal dermatitis, furunculosis, acne.

Special Senses: conjunctivitis, taste perversion, dry eyes.

Urinary: urinary tract infection, hematuria, vaginal moniliasis.

Patients With AIDS-Related Kaposi's Sarcoma

The safety data below is based on the experience reported in 753 patients with AIDS-related Kaposi's sarcoma enrolled in four studies. The median age of the population was 38.7 years (range 24-70 years), which was 99% male, 1% female, 88% Caucasian, 6% Hispanic, 4% Black, and 2% Asian/other/unknown. The majority of patients were treated with 20 mg/m^2 of DOXIL every two to three weeks. The median time on study was 127 days and ranged from 1 to 811 days. The median cumulative dose was 120 mg/m^2 and ranged from 3.3 to 798.6 mg/m^2. Twenty-six patients (3.0%) received cumulative doses of greater than 450 mg/m^2.

Of these 753 patients, 61.2% were considered poor risk for KS tumor burden, 91.5% poor for immune system, and 46.9% for systemic illness; 36.2% were poor risk for all three categories. Patients' median CD4 count was 21.0 $cells/mm^3$, with 50.8% of patients having less than 50 $cells/mm^3$. The mean absolute neutrophil count at study entry was approximately 3,000 $cells/mm^3$.

Patients received a variety of potentially myelotoxic drugs in combination with DOXIL. Of the 693 patients with concomitant medication information, 58.7% were on one or more antiretroviral medications; 34.9% patients were on zidovudine (AZT), 20.8% on didanosine (ddI), 16.5% on zalcitabine (ddC), and 9.5% on stavudine (D4T). A total of 85.1% patients were

on PCP prophylaxis, most (54.4%) on sulfamethoxazole/trimethoprim. Eighty-five percent of patients were receiving antifungal medications, primarily fluconazole (75.8%). Seventy-two percent of patients were receiving antivirals, 56.3% acyclovir, 29% ganciclovir, and 16% foscarnet. In addition, 47.8% patients received colony-stimulating factors (sargramostim/filgrastim) sometime during their course of treatment.

Adverse reactions led to discontinuation of treatment in 5% of patients with AIDS related Kaposi's sarcoma. Those that did so included bone marrow suppression, cardiac adverse reactions, infusion-related reactions, toxoplasmosis, HFS, pneumonia, cough/dyspnea, fatigue, optic neuritis, progression of a non-KS tumor, allergy to penicillin, and unspecified reasons.

Table 8: Hematology Data Reported in Patients With AIDS-Related Kaposi's Sarcoma

	Patients With Refractory or Intolerant AIDS-Related Kaposi's Sarcoma (n = 74)		Total Patients With AIDS-Related Kaposi's Sarcoma (n = 720)	
Neutropenia				
< 1000/mm^3	34	(45.9%)	352	(48.9%)
< 500/mm^3	8	(10.8%)	96	(13.3%)
Anemia				
< 10 g/dL	43	(58.1%)	399	(55.4%)
< 8 g/dL	12	(16.2%)	131	(18.2%)
Thrombocytopenia				
< 150,000/mm^3	45	(60.8%)	439	(60.9%)
< 25,000/mm^3	1	(1.4%)	30	(4.2%)

Table 9: Probably and Possibly Drug-Related Non-Hematologic Adverse Reactions Reported in ≥ 5% of Patients With AIDS-Related Kaposi's Sarcoma

Adverse Reactions	Patients With Refractory or Intolerant AIDS-Related Kaposi's Sarcoma (n = 77)		Total Patients With AIDS-Related Kaposi's Sarcoma (n = 705)	
Nausea	14	(18.2%)	119	(16.9%)
Asthenia	5	(6.5%)	70	(9.9%)
Fever	6	(7.8%)	64	(9.1%)
Alopecia	7	(9.1%)	63	(8.9%)
Alkaline Phosphatase Increase	1	(1.3%)	55	(7.8%)
Vomiting	6	(7.8%)	55	(7.8%)
Diarrhea	4	(5.2%)	55	(7.8%)
Stomatitis	4	(5.2%)	48	(6.8%)
Oral Moniliasis	1	(1.3%)	39	(5.5%)

The following additional (not in table) adverse reactions were observed in patients with AIDS-related Kaposi's sarcoma.

Incidence 1% to 5%

Body as a Whole: headache, back pain, infection, allergic reaction, chills.

Cardiovascular: chest pain, hypotension, tachycardia.

Cutaneous: herpes simplex, rash, itching.

Digestive: mouth ulceration, anorexia, dysphagia.

Metabolic and Nutritional: SGPT increase, weight loss, hyperbilirubinemia.

Other: dyspnea, pneumonia, dizziness, somnolence.

Incidence Less Than 1%

Body As A Whole: sepsis, moniliasis, cryptococcosis.

Cardiovascular: thrombophlebitis, cardiomyopathy, palpitation, bundle branch block, congestive heart failure, heart arrest, thrombosis, ventricular arrhythmia.

Digestive: hepatitis.

Metabolic and Nutritional Disorders: dehydration

Respiratory: cough increase, pharyngitis.

Skin and Appendages: maculopapular rash, herpes zoster.

Special Senses: taste perversion, conjunctivitis.

Patients With Multiple Myeloma

The safety data below are from 318 patients treated with DOXIL (30 mg/m^2 as a 1-hr i.v. infusion) administered on day 4 following bortezomib (1.3 mg/m^2 i.v. bolus on days 1, 4 , 8 and 11) every three weeks, in a randomized, open-label, multicenter study. In this study, patients in the DOXIL + bortezomib combination group were treated for a median number of 138 days (range 21-410 days). The population was 28-85 years of age, 58% male, 42% female, 90% Caucasian, 6% Black, and 4% Asian and other. Table 10 lists adverse reactions reported in 10% or more of patients treated with DOXIL in combination with bortezomib for multiple myeloma.

Table 10. Frequency of treatment emergent adverse reactions reported in ≥10% patients treated for multiple myeloma with DOXIL in combination with bortezomib, by Severity, Body System, and MedDRA Terminology.

Adverse Reaction	DOXIL + bortezomib (n=318)			Bortezomib (n=318)		
	Any (%)	Grade 3	Grade 4	Any (%)	Grade 3	Grade 4
Blood and lymphatic system disorders						
Neutropenia	36	22	10	22	11	5
Thrombocytopenia	33	11	13	28	9	8
Anemia	25	7	2	21	8	2
General disorders and administration site conditions						
Fatigue	36	6	1	28	3	0
Pyrexia	31	1	0	22	1	0
Asthenia	22	6	0	18	4	0
Gastrointestinal disorders						
Nausea	48	3	0	40	1	0
Diarrhea	46	7	0	39	5	0
Vomiting	32	4	0	22	1	0

Continued

Adverse Reaction	DOXIL + bortezomib (n=318)			Bortezomib (n=318)		
	Any (%)	Grade 3	Grade 4	Any (%)	Grade 3	Grade 4
Constipation	31	1	0	31	1	0
Mucositis/Stomatitis	20	2	0	5	<1	0
Abdominal pain	11	1	0	8	1	0
Infections and infestations						
Herpes zoster	11	2	0	9	2	0
Herpes simplex	10	0	0	6	1	0
Investigations						
Weight decreased	12	0	0	4	0	0
Metabolism and Nutritional disorders						
Anorexia	19	2	0	14	<1	0
Nervous system disorders						
Peripheral Neuropathy*	42	7	<1	45	10	1
Neuralgia	17	3	0	20	4	1
Paresthesia/dysesthesia	13	<1	0	10	0	0
Respiratory, thoracic and mediastinal disorders						
Cough	18	0	0	12	0	0
Skin and subcutaneous tissue disorders						
Rash**	22	1	0	18	1	0
Hand-foot syndrome	19	6	0	<1	0	0

*Peripheral neuropathy includes the following adverse reactions: peripheral sensory neuropathy, neuropathy peripheral, polyneuropathy, peripheral motor neuropathy, and neuropathy NOS.

**Rash includes the following adverse reactions: rash, rash erythematous, rash macular, rash maculo-papular, rash pruritic, exfoliative rash, and rash generalized.

6.3 Post Marketing Experience

The following additional adverse reactions have been identified during post approval use of DOXIL. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

Musculoskeletal and Connective Tissue Disorders: rare cases of muscle spasms.

Respiratory, Thoracic and Mediastinal Disorders: rare cases of pulmonary embolism (in some cases fatal).

Hematologic disorders: Secondary acute myelogenous leukemia with and without fatal outcome has been reported in patients whose treatment included DOXIL.

Skin and subcutaneous tissue disorders: rare cases of erythema multiforme, Stevens-Johnson syndrome and toxic epidermal necrolysis have been reported.

7 DRUG INTERACTIONS

No formal drug interaction studies have been conducted with DOXIL. DOXIL may interact with drugs known to interact with the conventional formulation of doxorubicin HCl.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category D *[see Warnings and Precautions (5.6)]*.

DOXIL is embryotoxic at doses of 1 mg/kg/day in rats and is embryotoxic and abortifacient at 0.5 mg/kg/day in rabbits (both doses are about one-eighth the 50 mg/m^2 human dose on a mg/m^2 basis). Embryotoxicity was characterized by increased embryo-fetal deaths and reduced live litter sizes.

8.3 Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs, including anthracyclines, are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from DOXIL, mothers should discontinue nursing prior to taking this drug.

8.4 Pediatric Use

The safety and effectiveness of DOXIL in pediatric patients have not been established.

8.5 Geriatric Use

Of the patients treated with DOXIL in the randomized ovarian cancer study, 34.7% (n=83) were 65 years of age or older while 7.9% (n=19) were 75 years of age or older. Of the 318 patients treated with DOXIL in combination with bortezomib for multiple myeloma, 37% were 65 years of age or older and 8% were 75 years of age or older. No overall differences in safety or efficacy were observed between these patients and younger patients.

8.6 Hepatic Impairment

The pharmacokinetics of DOXIL has not been adequately evaluated in patients with hepatic impairment. Doxorubicin is eliminated in large part by the liver. Thus, DOXIL dosage should be reduced in patients with impaired hepatic function *[see Dosage and Administration (2.6)]*.

Prior to DOXIL administration, evaluation of hepatic function is recommended using conventional clinical laboratory tests such as SGOT, SGPT, alkaline phosphatase, and bilirubin *[see Dosage and Administration (2.6)]*.

10 OVERDOSAGE

Acute overdosage with doxorubicin HCl causes increases in mucositis, leucopenia, and thrombocytopenia.

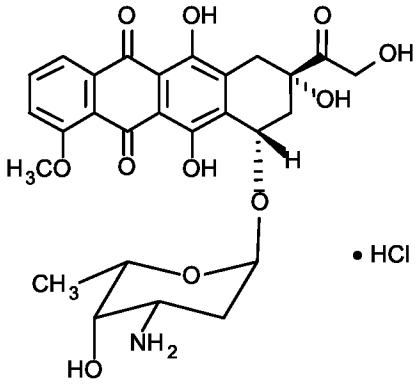
Treatment of acute overdosage consists of treatment of the severely myelosuppressed patient with hospitalization, antibiotics, platelet and granulocyte transfusions, and symptomatic treatment of mucositis.

11 DESCRIPTION

DOXIL (doxorubicin HCl liposome injection) is doxorubicin hydrochloride (HCl) encapsulated in STEALTH® liposomes for intravenous administration.

Doxorubicin is an anthracycline topoisomerase inhibitor isolated from *Streptomyces peucetius* var. *caesius*.

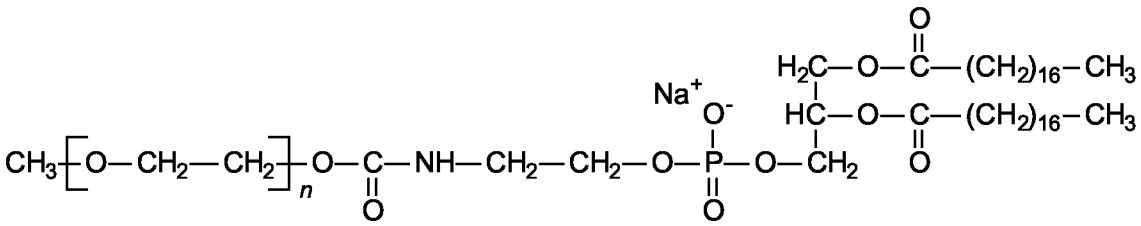
Doxorubicin HCl, which is the established name for (8S,10S)-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-8-glycolyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride, has the following structure:



The molecular formula of the drug is C27 H29 NO11·HCl; its molecular weight is 579.99.

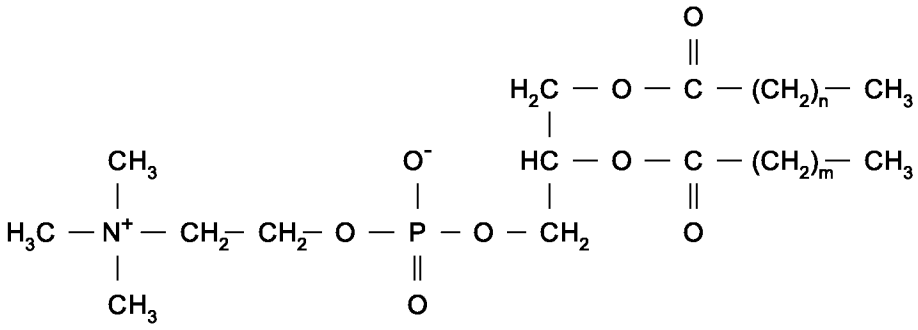
DOXIL is provided as a sterile, translucent, red liposomal dispersion in 10-mL or 30-mL glass, single use vials. Each vial contains 20 mg or 50 mg doxorubicin HCl at a concentration of 2 mg/mL and a pH of 6.5. The STEALTH® liposome carriers are composed of N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (MPEG-DSPE), 3.19 mg/mL; fully hydrogenated soy phosphatidylcholine (HSPC), 9.58 mg/mL; and cholesterol, 3.19 mg/mL. Each mL also contains ammonium sulfate, approximately 2 mg; histidine as a buffer; hydrochloric acid and/or sodium hydroxide for pH control; and sucrose to maintain isotonicity. Greater than 90% of the drug is encapsulated in the STEALTH® liposomes.

MPEG-DSPE has the following structural formula:



n = ca. 45

HSPC has the following structural formula:



m, n = 14 or 16

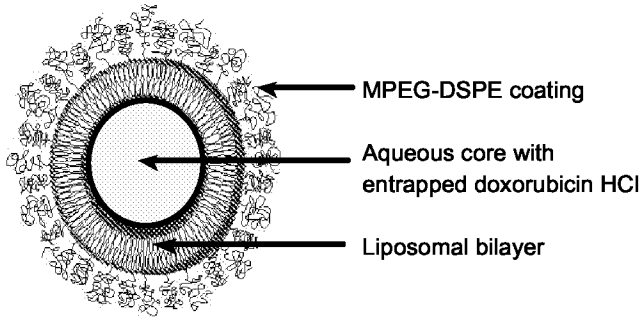
12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

The active ingredient of DOXIL is doxorubicin HCl. The mechanism of action of doxorubicin HCl is thought to be related to its ability to bind DNA and inhibit nucleic acid synthesis. Cell structure studies have demonstrated rapid cell penetration and perinuclear chromatin binding, rapid inhibition of mitotic activity and nucleic acid synthesis, and induction of mutagenesis and chromosomal aberrations.

DOXIL is doxorubicin HCl encapsulated in long-circulating STEALTH® liposomes. Liposomes are microscopic vesicles composed of a phospholipid bilayer that are capable of encapsulating active drugs. The STEALTH® liposomes of DOXIL are formulated with surface-bound methoxypolyethylene glycol (MPEG), a process often referred to as pegylation, to protect liposomes from detection by the mononuclear phagocyte system (MPS) and to increase blood circulation time.

Representation of a STEALTH® liposome:



STEALTH® liposomes have a half-life of approximately 55 hours in humans. They are stable in blood, and direct measurement of liposomal doxorubicin shows that at least 90% of the drug (the assay used cannot quantify less than 5-10% free doxorubicin) remains liposome-encapsulated during circulation.

It is hypothesized that because of their small size (ca. 100 nm) and persistence in the circulation, the pegylated DOXIL liposomes are able to penetrate the altered and often compromised vasculature of tumors. This hypothesis is supported by studies using colloidal gold-containing STEALTH® liposomes, which can be visualized microscopically. Evidence of penetration of STEALTH® liposomes from blood vessels and their entry and accumulation in tumors has been seen in mice with C-26 colon carcinoma tumors and in transgenic mice with Kaposi's sarcoma-like lesions. Once the STEALTH® liposomes distribute to the tissue compartment, the encapsulated doxorubicin HCl becomes available. The exact mechanism of release is not understood.

12.3 Pharmacokinetics

The plasma pharmacokinetics of DOXIL were evaluated in 42 patients with AIDS-related Kaposi's sarcoma (KS) who received single doses of 10 or 20 mg/m^2 administered by a 30-minute infusion. Twenty-three of these patients received single doses of both 10 and 20 mg/m^2 with a 3-week wash-out period between doses. The pharmacokinetic parameter values of DOXIL, given for total doxorubicin (mostly liposomally bound), are presented in Table 11.

Table 11: Pharmacokinetic Parameters of DOXIL in Patients With AIDS-Related Kaposi's Sarcoma

	Dose	
Parameter (units)	10 mg/m^2	20 mg/m^2
Peak Plasma Concentration (μg/mL)	4.12 ± 0.215	8.34 ± 0.49
Plasma Clearance (L/h/m^2)	0.056 ± 0.01	0.041 ± 0.004
Steady State Volume of Distribution (L/m^2)	2.83 ± 0.145	2.72 ± 0.120
AUC (μg/mL•h)	277 ± 32.9	590 ± 58.7
First Phase (λ_1) Half-Life (h)	4.7 ± 1.1	5.2 ± 1.4
Second Phase (λ_1) Half-Life (h)	52.3 ± 5.6	55.0 ± 4.8

N = 23
Mean ± Standard Error

DOXIL displayed linear pharmacokinetics over the range of 10 to 20 mg/m^2. Disposition occurred in two phases after DOXIL administration, with a relatively short first phase (≈ 5 hours) and a prolonged second phase (≈ 55 hours) that accounted for the majority of the area under the curve (AUC).

The pharmacokinetics of DOXIL at a 50 mg/m^2 dose is reported to be nonlinear. At this dose, the elimination half-life of DOXIL is expected to be longer and the clearance lower compared to a 20 mg/m^2 dose. The exposure (AUC) is thus expected to be more than proportional at a 50 mg/m^2 dose when compared with the lower doses.

Distribution:

In contrast to the pharmacokinetics of doxorubicin, which displays a large volume of distribution, ranging from 700 to 1100 L/m^2, the small steady state volume of distribution of DOXIL shows that DOXIL is confined mostly to the vascular fluid volume. Plasma protein binding of DOXIL has not been determined; the plasma protein binding of doxorubicin is approximately 70%.

Metabolism:

Doxorubicinol, the major metabolite of doxorubicin, was detected at very low levels (range: of 0.8 to 26.2 ng/mL) in the plasma of patients who received 10 or 20 mg/m^2 DOXIL.

Excretion:

The plasma clearance of DOXIL was slow, with a mean clearance value of 0.041 L/h/m^2 at a dose of 20 mg/m^2. This is in contrast to doxorubicin, which displays a plasma clearance value ranging from 24 to 35 L/h/m^2.

Because of its slower clearance, the AUC of DOXIL, primarily representing the circulation of liposome-encapsulated doxorubicin, is approximately two to three orders of magnitude larger than the AUC for a similar dose of conventional doxorubicin HCl as reported in the literature.

Special Populations:

The pharmacokinetics of DOXIL have not been separately evaluated in women, in members of different ethnic groups, or in individuals with renal or hepatic insufficiency.

Drug-Drug Interactions:

Drug-drug interactions between DOXIL and other drugs, including antiviral agents, have not been adequately evaluated in patients with ovarian cancer, AIDS-related Kaposi's sarcoma or multiple myeloma.

Tissue Distribution in Patients with Kaposi's Sarcoma:

Kaposi's sarcoma lesions and normal skin biopsies were obtained at 48 and 96 hours post infusion of 20 mg/m^2 DOXIL in 11 patients. The concentration of DOXIL in KS lesions was a median of 19 (range, 3-53) times higher than in normal skin at 48 hours post treatment; however, this was not corrected for likely differences in blood content between KS lesions and normal skin. The corrected ratio may lie between 1 and 22 times. Thus, higher concentrations of DOXIL are delivered to KS lesions than to normal skin.

13 NON-CLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, and Impairment of Fertility

Although no studies have been conducted with DOXIL, doxorubicin HCl and related compounds have been shown to have mutagenic and carcinogenic properties when tested in experimental models.

STEALTH® liposomes without drug were negative when tested in Ames, mouse lymphoma and chromosomal aberration assays *in vitro*, and mammalian micronucleus assay *in vivo*.

The possible adverse effects on fertility in males and females in humans or experimental animals have not been adequately evaluated. However, DOXIL resulted in mild to moderate ovarian and testicular atrophy in mice after a single dose of 36 mg/kg (about twice the 50 mg/m^2 human dose on a mg/m^2 basis). Decreased testicular weights and hypospermia were present in rats after repeat doses $\geq$ 0.25 mg/kg/day (about one thirtieth the 50 mg/m^2 human dose on a mg/m^2 basis), and diffuse degeneration of the seminiferous tubules and a marked decrease in spermatogenesis were observed in dogs after repeat doses of 1 mg/kg/day (about one half the 50 mg/m^2 human dose on a mg/m^2 basis).

14 CLINICAL STUDIES

14.1 Ovarian Cancer

DOXIL (doxorubicin HCl liposome injection) was studied in three open-label, single-arm, clinical studies of 176 patients with metastatic ovarian cancer. One hundred forty-five (145) of these patients were refractory to both paclitaxel- and platinum-based chemotherapy regimens.

Refractory ovarian cancer is defined as disease progression while on treatment, or relapse within 6 months of completing treatment. Patients in these studies received DOXIL at 50 mg/m^2 infused over one hour every 3 or 4 weeks for 3-6 cycles or longer in the absence of dose-limiting toxicity or progression of disease.

The baseline demographics and clinical characteristics of the patients with refractory ovarian cancer are provided in **Table 12** below.

Table 12: Patient Demographics for Patients With Refractory Ovarian Cancer From Single Arm Ovarian Cancer Studies

	Study 1 (U.S.) (n = 27)	Study 2 (U.S.) (n = 82)	Study 3 (non-U.S.) (n = 36)
Age at Diagnosis (Years)			
Median	64	61.5	51.5
Range	46 – 75	34 – 85	22 – 80
Drug-Free Interval (Months)			
Median	1.8	1.7	2.6
Range	0.5 – 15.6	0.6 – 7.0	0.7 – 15.2
Sum of Lesions at Baseline (cm^2)			
Median	25	18.3	32.4
Range	1.2 – 230.0	1.3 – 285.0	0.3 – 114.0
FIGO Staging			
I	1 (3.7%)	3 (3.7%)	4 (11.1%)
II	3 (11.1%)	3 (3.7%)	1 (2.8%)
III	15 (55.6%)	60 (73.2%)	24 (66.7%)
IV	8 (29.6%)	16 (19.5%)	6 (16.7%)
Not Specified	—	—	1 (2.8%)
CA-125 at Baseline			
Median	123.5	199.0	1004.5
Range	20 – 14,012	7 – 46,594	20 – 12,089
Number of Prior Chemotherapy Regimens			
1	7 (25.9%)	13 (15.9%)	9 (25.0%)
2	11 (40.7%)	44 (53.7%)	19 (52.8%)
3	6 (22.2%)	25 (30.5%)	8 (22.8%)
4	3 (11.1%)	—	—

The primary efficacy parameter was response rate for the population of patients refractory to both paclitaxel- and a platinum-containing regimen. Assessment of response was based on Southwest Oncology Group (SWOG) criteria, and required confirmation four weeks after the initial observation. Secondary efficacy parameters were time to response, duration of response, and time to progression.

The response rates for the individual single arm studies are given in **Table 13** below.

Table 13: Response Rates in Patients With Refractory Ovarian Cancer From Single Arm Ovarian Cancer Studies

	Study 1 (U.S.)	Study 2 (U.S.)	Study 3 (non-U.S.)
Response Rate	22.2% (6/27)	17.1% (14/82)	0% (0/36)
95% Confidence Interval	8.6% - 42.3%	9.7% - 27.0%	0.0% - 9.7%

When the data from the single arm studies are combined, the response rate for all patients refractory to paclitaxel and platinum agents was 13.8% (20/145) (95% CI 8.1% to 19.3%). The median time to progression was 15.9 weeks, the median time to response was 17.6 weeks, and the duration of response was 39.4 weeks.

DOXIL (doxorubicin HCl liposome injection) was also studied in a randomized, multicenter, open-label, study in 474 patients with epithelial ovarian cancer after platinum-based chemotherapy. Patients in this study received an initial dose of either DOXIL 50 mg/m^2 infused over one hour every 4 weeks or topotecan 1.5 mg/m^2 infused daily for 5 consecutive days every 3 weeks. Patients were stratified according to platinum sensitivity and the presence of bulky disease (presence of tumor mass greater than 5 cm in size). Platinum sensitivity is defined by response to initial platinum-based therapy and a progression-free interval of greater than 6 months off treatment. The primary efficacy endpoint for this study was time to progression (TTP). Other efficacy endpoints included overall survival and objective response rate.

The baseline patient demographic and clinical characteristics are provided in **Table 14** below.

Table 14: Ovarian Cancer Randomized Study Baseline Demographic and Clinical Characteristics

	DOXIL (n = 239)	Topotecan (n = 235)
Age at Diagnosis (Years)		
Median	60.0	60.0
Range	27 – 87	25 – 85
Drug-Free Interval (Months)		
Median	7.0	6.7
Range	0.9 – 82.1	0.5 – 109.6
FIGO Staging		
I	11 (4.6%)	15 (6.4%)
II	13 (5.4%)	8 (3.4%)
III	175 (73.2%)	164 (69.8%)
IV	40 (16.7%)	48 (20.4%)
Platinum Sensitivity		
Sensitive	109 (45.6%)	110 (46.8%)
Refractory	130 (54.4%)	125 (53.2%)
Bulky Disease		
Present	108 (45.2%)	105 (44.7%)
Absent	131 (54.8%)	130 (55.3%)

Study results are provided in **Table 15**.

There was no statistically significant difference in TTP between the two treatment arms.

Table 15: Results of Efficacy Analyses[a]

	Protocol Defined ITT Population	
	DOXIL (n = 239)	Topotecan (n = 235)
TTP (Protocol Specified Primary Endpoint)		
Median (Months)[b]	4.1	4.2
p-value[c]	0.617	
Hazard Ratio[d]	0.955	
95% CI for Hazard Ratio	(0.762, 1.196)	
Overall Survival		
Median (Months)[b]	14.4	13.7
p-value*	0.05	
Hazard Ratio[d]	0.822	
95% CI for Hazard Ratio	(0.676, 1.000)	
Response Rate		
Overall Response n (%)	47 (19.7)	40 (17.0)
Complete Response n (%)	9 (3.8)	11 (4.7)
Partial Response n (%)	38 (15.9)	29 (12.3)
Median Duration of Response (Months)[b]	6.9	5.9

[a] Analysis based on investigators' strata for protocol defined ITT population.
[b] Kaplan-Meier estimates.
[c] p-value is based on the stratified log-rank test.
[d] Hazard ratio is based on Cox proportional-hazard model with the treatment as single independent variable. A hazard ratio less than 1 indicates an advantage for DOXIL.
* p-value not adjusted for multiple comparisons.

14.2 AIDS-Related Kaposi's Sarcoma

DOXIL was studied in an open-label, single-arm, multicenter study utilizing DOXIL at 20 mg/m^2 by intravenous infusion every three weeks, generally until progression or intolerance occurred. In an interim analysis, the treatment history of 383 patients was reviewed, and a cohort of 77 patients was retrospectively identified as having disease progression on prior systemic combination chemotherapy (at least 2 cycles of a regimen containing at least two of three treatments: bleomycin, vincristine or vinblastine, or doxorubicin) or as being intolerant to such therapy. Forty-nine of the 77 (64%) patients had received prior doxorubicin HCl.

These 77 patients were predominantly Caucasian, homosexual males with a median CD4 count of 10 cells/mm^3. Their age ranged from 24 to 54 years, with a mean age of 38 years. Using the ACTG staging criteria, 78% of the patients were at poor risk for tumor burden, 96% at poor risk for immune system, and 58% at poor risk for systemic illness at baseline. Their mean Karnofsky status score was 74%. All 77 patients had cutaneous or subcutaneous lesions, 40% also had oral lesions, 26% pulmonary lesions, and 14% of patients had lesions of the stomach/intestine.

The majority of these patients had disease progression on prior systemic combination chemotherapy.

The median time on study for these 77 patients was 155 days and ranged from 1 to 456 days. The median cumulative dose was 154 mg/m^2 and ranged from 20 to 620 mg/m^2.

Two analyses of tumor response were used to evaluate the effectiveness of DOXIL: one analysis based on investigator assessment of changes in lesions over the entire body, and one analysis based on changes in indicator lesions.

Investigator Assessment

Investigator response was based on modified ACTG criteria. Partial response was defined as no new lesions, sites of disease, or worsening edema; flattening of ≥50% of previously raised lesions or area of indicator lesions decreasing by ≥50%; and response lasting at least 21 days with no prior progression.

Indicator Lesion Assessment

A retrospectively defined analysis was conducted based on assessment of the response of up to five prospectively identified representative indicator lesions. A partial response was defined as flattening of ≥50% of previously raised indicator lesions, or >50% decrease in the area of indicator lesions and lasting at least 21 days with no prior progression.

Only patients with adequate documentation of baseline status and follow-up assessments were considered evaluable for response. Patients who received concomitant KS treatment during study, who completed local radiotherapy to sites encompassing one or more of the indicator lesions within two months of study entry, who had less than four indicator lesions, or who had less than three raised indicator lesions at baseline (the latter applies solely to indicator lesion assessment) were considered nonevaluable for response. Of the 77 patients who had disease progression on prior systemic combination chemotherapy or who were intolerant to such therapy, 34 were evaluable for investigator assessment and 42 were evaluable for indicator lesion assessment.

Table 16: Response in Patients with Refractory[a] AIDS-related Kaposi's Sarcoma

Investigator Assessment	All Evaluable Patients (n = 34)	Evaluable Patients Who Received Prior Doxorubicin (n = 20)
Response[b]		
Partial (PR)	27%	30%
Stable	29%	40%
Progression	44%	30%
Duration of PR (Days)		
Median	73	89
Range	42+ - 210+	42+ - 210+
Time to PR (Days)		
Median	43	53
Range	15 – 133	15 – 109

Indicator Lesion Assessment	All Evaluable Patients (n = 42)	Evaluable Patients Who Received Prior Doxorubicin (n = 23)
Response[b]		
Partial (PR)	48%	52%
Stable	26%	30%
Progression	26%	17%
Duration of PR (Days)		
Median	71	79
Range	22+ - 210+	35 - 210+
Time to PR (Days)		
Median	22	48
Range	15 – 109	15 – 109

[a] Patients with disease that progressed on prior combination chemotherapy or who were intolerant to such therapy.
[b] There were no complete responses in this population.

Retrospective efficacy analyses were performed on two studies that had subsets of patients who received single agent DOXIL and who were on stable antiretroviral therapy for at least 60 days prior to enrollment and at least until a response was demonstrated. In one cooperative group trial that was closed early due to slow accrual, 7 of 17 patients (40%) on stable antiretroviral therapy had a durable response. The median duration was not reached but was longer than 11.6 months. In another trial, 4 of 11 patients (40%) on stable antiretroviral therapy demonstrated durable responses.

14.3 Multiple Myeloma

The safety and efficacy of DOXIL in combination with bortezomib in the treatment of multiple myeloma were evaluated in a randomized, open label, international multicenter study. This study included 646 patients who have not previously received bortezomib and whose disease progressed during or after at least one prior therapy. Patients were randomized (1:1 ratio)

to receive either DOXIL (30 mg/m^2 as a 1-hr i.v. infusion) administered on day 4 following bortezomib (1.3 mg/m^2 i.v. bolus on days 1, 4 , 8 and 11) or bortezomib alone (1.3 mg/m^2 i.v. bolus on days 1, 4 , 8 and 11). Treatment was administered every 3 weeks. Patients were treated for up to 8 cycles until disease progression or the occurrence of unacceptable toxicity. Patients who maintained a response were allowed to receive further treatment. The median number of cycles in each treatment arm was 5 (range 1-18). The baseline demographics and clinical characteristics of the patients with multiple myeloma are provided in **Table 17** below.

Table 17 Summary of Baseline Patient and Disease Characteristics

Patient Characteristics	**DOXIL + bortezomib n=324**	**bortezomib n=322**
Median age in years (range)	61 (28, 85)	62 (34, 88)
% Male/female	58 / 42	54 / 46
% Caucasian/Black/other	90 / 6/ 4	94 / 4 / 2%
Disease Characteristics		
% with IgG/IgA/Light chain	57 / 27 / 12	62 / 24 /11
% β_2-microglobulin group		
≤2.5 mg/L	14	14
>2.5 mg/L and ≤5.5 mg/L	56	55
>5.5 mg/L	30	31
Serum M-protein (g/dL): Median (Range)	2.5 (0-10.0)	2.7 (0-10.0)
Urine M-protein (mg/24 hours): Median (Range)	107 (0-24883)	66 (0-39657)
Median Months Since Diagnosis	35.2	37.5
% Prior Therapy		
One	34	34
More than one	66	66
Prior Systemic Therapies for Multiple Myeloma		
Corticosteroid (%)	99	>99
Anthracyclines	68	67
Alkylating agent (%)	92	90
Thalidomide/lenalidomide (%)	40	43
Stem cell transplantation (%)	57	54

The primary endpoint in this study was time to progression (TTP). TTP was defined as the time from randomization to the first occurrence of progressive disease or death due to progressive disease. The combination arm demonstrated significant improvement in TTP. As the prespecified primary objective was achieved at the interim analysis, patients in the bortezomib monotherapy group were then allowed to receive the DOXIL + bortezomib combination. Survival continued to be followed after the interim analysis and survival data are not mature at this time. Efficacy results are as shown in **Table 18 and Figure 1.**

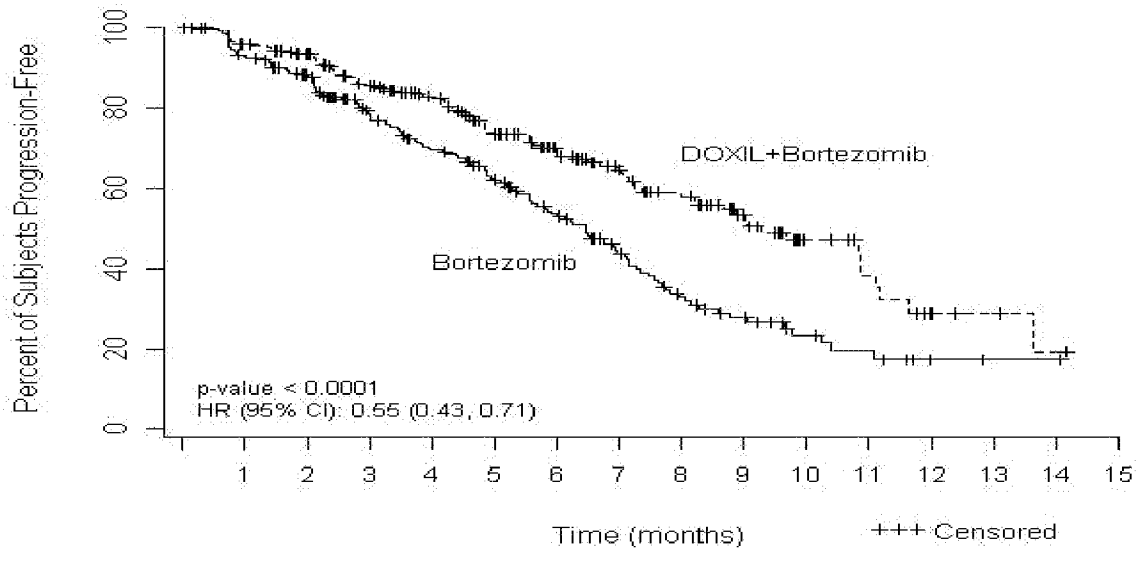
Table 18 Efficacy of DOXIL in combination with bortezomib in the treatment of patients with multiple myeloma

Endpoint	DOXIL + bortezomib n=324		Bortezomib n=322
Time to Progression[a]			
Progression or death due to progression (n)	99		150
Censored (n)	225		172
Median in days (months)	282 (9.3)		197 (6.5)
95% CI	250;338		170;217
Hazard ratio[b]		0.55	
(95% CI)		(0.43, 0.71)	
p-value[c]		<0.0001	
Response (n)[d]	303		310
% Complete Response (CR)	5		3
%Partial Response (PR)	43		40
%CR + PR	48		43
p-value[e]		0.251	
Median Duration of Response (months)	10.2		7.0
(95% CI)	(10.2;12.9)		(5.9;8.3)

[a] Kaplan Meier estimate.
[b] Hazard ratio based on stratified Cox proportional hazards regression. A hazard ratio < 1 indicates an advantage for DOXIL+bortezomib.
[c] Stratified log-rank test.
[d] RR as per EBMT criteria.
[e] Cochran-Mantel-Haenszel test adjusted for the stratification factors.

Time to progression outcomes were consistent with the overall result across most subgroups defined by patient demographic and baseline characteristics. There were too few Blacks or Asian patients to adequately assess differences in effects for the race subgroup.

Figure 1- Time to Progression Kaplan-Meier Curve



Number of Subjects at Risk

DOXIL+Bortezomib	324	301	269	201	170	127	97	70	56	38	19	13	6	4	2	0
Bortezomib	322	290	253	189	150	112	84	56	35	25	14	9	2	1	1	0

15 REFERENCES

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16 HOW SUPPLIED/STORAGE AND HANDLING

DOXIL (doxorubicin HCl liposome injection) is supplied as a sterile, translucent, red liposomal dispersion in 10-mL or 30-mL glass, single use vials.

Each 10-mL vial contains 20 mg doxorubicin HCl at a concentration of 2 mg/mL.

Each 30-mL vial contains 50 mg doxorubicin HCl at a concentration of 2 mg/mL.

Refrigerate unopened vials of DOXIL at 2°-8°C (36°-46°F). Avoid freezing. Prolonged freezing may adversely affect liposomal drug products; however, short-term freezing (less than 1 month) does not appear to have a deleterious effect on DOXIL.

The following packages of six individually cartoned vials are available:

Table 19

mg in vial	fill volume	vial size	NDC #s
20 mg vial	10-mL	10-mL	17314-9600-1
50 mg vial	25-mL	30-mL	17314-9600-2

17 PATIENT COUNSELING INFORMATION

Patients and patients' caregivers should be informed of the expected adverse effects of DOXIL, particularly hand-foot syndrome, stomatitis, and neutropenia and related complications of neutropenic fever, infection, and sepsis.

Hand-Foot Syndrome (HFS): Patients who experience tingling or burning, redness, flaking, bothersome swelling, small blisters, or small sores on the palms of their hands or soles of their feet (symptoms of Hand-Foot Syndrome) should notify their physician.

Stomatitis: Patients who experience painful redness, swelling, or sores in the mouth (symptoms of stomatitis) should notify their physician.

Fever and Neutropenia: Patients who develop a fever of 100.5°F or higher should notify their physician.

Nausea, vomiting, tiredness, weakness, rash, or mild hair loss: Patients who develop any of these symptoms should notify their physician.

Following its administration, DOXIL may impart a reddish-orange color to the urine and other body fluids. This nontoxic reaction is due to the color of the product and will dissipate as the drug is eliminated from the body.

Manufactured by:
Ben Venue Laboratories, Inc.
Bedford, OH 44146

Distributed by:

Ortho Biotech Products, LP Raritan, NJ 08869-0670

{Package Eng.: To Add OBI Logo Here}

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/s/

Robert Justice
6/10/2008 08:20:22 PM

Development of a Highly Active Nanoliposomal Irinotecan Using a Novel Intraliposomal Stabilization Strategy

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Abstract

Liposome formulations of camptothecins have been actively pursued because of the potential for significant pharmacologic advantages from successful drug delivery of this important class of anticancer drugs. We describe nanoliposomal CPT-11, a novel nanoparticle/liposome construct containing CPT-11 (irinotecan) with unprecedented drug loading efficiency and *in vivo* drug retention. Using a modified gradient loading method featuring a sterically hindered amine with highly charged, multivalent anionic trapping agents, either polymeric (polyphosphate) or nonpolymeric (sucrose octasulfate), liposomes were capable of entrapping CPT-11 at extremely high drug-to-lipid ratios (>800 g CPT-11/mol phospholipid) and retaining encapsulated drug *in vivo* with a half-life of drug release in the circulation of 56.8 hours. CPT-11 was also protected from hydrolysis to the inactive carboxylate form and from metabolic conversion to SN-38 while circulating. The maximum tolerated dose in normal mice was determined to be 80 mg/kg for free CPT-11 and >320 mg/kg for nanoliposomal CPT-11. Nanoliposomal CPT-11 showed markedly superior efficacy when compared with free CPT-11 in human breast (BT474) and colon (HT29) cancer xenograft models. This study shows that intraliposomal stabilization of CPT-11 using a polymeric or highly charged, nonpolymeric polyanionic trapping agent results in a markedly active antitumor agent with low toxicity. (Cancer Res 2006; 66(6): 3271-7)

Introduction

Liposome-based systems have been used to enhance efficacy and/or ameliorate toxicity of certain drugs (1, 2). Thus far, the most successful approach has involved constructs engineered for long circulation times, combined with stable encapsulation of the active compound within the liposome; this allows liposomes to accumulate at sites of cancer, followed by intratumoral drug release. An example is PEGylated liposomal doxorubicin (3), which has received Food and Drug Administration approval for cancer treatment. However, the successful case of liposomal anthracyclines has not yet been matched by liposome constructs containing other anticancer drug classes, although recent progress has been made with vincristine (4–6) and certain camptothecin analogues (7–9). One of the key reasons for this has been the technical facility with which anthracyclines can be stably encapsulated in the liposome interior using remote-loading methodologies (10, 11), giving rise to stable liposome formulations that have been difficult to replicate with other classes of drugs.

CPT-11 {irinotecan; 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycampothecin} is a water-soluble camptothecin derivative currently used in cancer chemotherapy. The pharmacology of CPT-11 is complex, with extensive metabolic conversions involved in the activation, inactivation, and elimination of the drug (12, 13). CPT-11 is a prodrug that is converted by nonspecific carboxylesterases into a 100- to 1,000-fold more active metabolite, SN-38 (14). SN-38 is cleared via glucuronidation, for which major pharmacogenetic differences have been shown (15), and biliary excretion. In addition, CPT-11 and other camptothecins exist in a pH- and serum protein-dependent equilibrium between an active lactone form of the drug (predominant under acidic conditions) and an inactive carboxylate form (predominant at neutral or basic pH; ref. 16). These drug properties contribute to the marked heterogeneities in efficacy and toxicity observed clinically with CPT-11 (12, 17). Hence, drug carrier technologies represent a rational strategy to improve the pharmacokinetics and biodistribution of CPT-11 while protecting it from premature metabolism.

In this report, we describe a novel intraliposomal drug stabilization technology for encapsulation of CPT-11 into long-circulating liposome-based nanoparticles with high drug load and high *in vivo* stability, matching or surpassing previous liposomal drugs. This was achieved using polymeric or nonpolymeric highly charged anions, polyphosphate or sucrose octasulfate, as intraliposomal trapping agents in conjunction with a high-pK_a polyalkylamine gradient. The approach also allowed for preservation of the drug in its active lactone form within the liposome interior, protecting it from hydrolysis as well as premature conversion to SN-38. Here we use the term "nanoliposomal drug" to describe a nanoparticle consisting of a lipid bilayer scaffold encapsulating a nanoscale drug complex or aggregate that facilitates *in vivo* drug retention.

Materials and Methods

Liposome Preparation and Drug Loading

Solutions of triethylammonium salts of a linear poly(phosphate) (TEA-Pn, 13-18 phosphate units; Sigma Corp., St. Louis, MO) and sucrose octasulfate (TEA_8SOS) were prepared from commercially obtained sodium salts (Toronto Research Chemicals, Inc., North York, Ontario, Canada) by ion-exchange chromatography on the Dowex 50Wx8-200 resin in the H^+ form, immediately followed by titration with neat triethylamine. Residual sodium in either solution, as determined by potentiometry using a Na^+-selective electrode, was <1% of the cation content. Phosphate content was determined by inorganic phosphate assay following acid hydrolysis and was adjusted to 0.55 mol/L for TEA-Pn (osmolality, 430-480 mmol/kg). The TEA concentration was calculated from the amount of added TEA and was

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adjusted to 0.65 mol/L for TEA_8SOS solution (osmolality, 480-530 mmol/kg). The final pH for both solutions was 5.5 to 6.0.

Distearoylphosphatidylcholine (3 mol. parts), methoxypoly(ethylene)glycol (PEG2000)-derivatized distearoylphosphatidylethanolamine (0.015 mol. parts; Avanti Polar Lipids, Alabaster, AL), and cholesterol (2 mol. parts; Calbiochem, La Jolla, CA) were combined in ~50% (w/v) ethanolic solution and mixed with 10 volumes of the solution of TEA-Pn or TEA_8SOS at 60°C to 65°C. For pharmacokinetic studies, a nonexchangeable lipid label, [^{3}H] cholesteryl hexadecyl ether (Perkin-Elmer, Boston, MA), was added to the lipids in the amount of 0.5 mCi/mmol phospholipid. The lipid suspension was extruded 15 times through two stacked polycarbonate membranes (Nucleopore, Corning-Costar, Acton, MA) with 0.08-μm pore size using argon pressure at 60°C to 65°C. The extruded liposomes were 88 to 95 nm in diameter by dynamic light scattering.

Unentrapped triethylammonium polyanions were removed by chromatography on a Sepharose CL-4B size exclusion column eluted with HEPES-buffered dextrose (5 mmol/L HEPES, 5% dextrose, pH 6.5). CPT-11·HCl (kindly provided by TTY Biopharmaceuticals, Taipei, Taiwan) was added to the liposomes at a ratio of 500 g CPT-11/mol phospholipid and the pH adjusted to 6.5. The resulting solution was heated to 60°C for 30 minutes and then quenched on ice for 15 minutes. Unencapsulated CPT-11 was subsequently removed using a Sephadex G-75 column eluted with HEPES-buffered saline (5 mmol/L HEPES, 145 mmol/L NaCl, pH 6.5). The loading efficiency was determined in all preparations by quantitating both drug and phospholipid and comparing the resulting drug/phospholipid ratio to its input value. CPT-11 was determined spectrophotometrically at 372 nm in acid/methanol (20 volume % 0.5 mol/L phosphoric acid/80 volume % methanol). Phospholipid was quantitated using a standard phosphate assay (18).

Pharmacokinetic Studies

Female Sprague-Dawley rats (190-210 g) with indwelling central venous catheters were injected with a 0.2 to 0.3 mL bolus of ^{3}H-CHE-labeled CPT-11 liposomes (10 mg/kg). Blood samples (0.2-0.3 mL) were drawn at various times postinjection using a heparin-treated syringe. The withdrawn blood volume was replaced using PBS. Blood samples were diluted with 0.3 mL of ice-cold PBS containing 0.04% EDTA, weighed, and centrifuged. Plasma was assayed for CPT-11 [by fluorometry or high-performance liquid chromatography (HPLC)] and for liposome label (scintillation radioactivity counting). The percent of drug remaining in the liposomes was calculated by dividing the drug/lipid ratio in the blood samples by that of the injected liposomes (taken as 100%). Because free CPT-11 is cleared at a much faster rate than liposomes (Fig. 3*A*), a change in the CPT-11-to-liposomal lipid ratio was indicative of drug leakage from the carrier. Noncompartmental pharmacokinetics data analysis was done using PK Solutions 2.0 software (Summit Research Services, Montrose, CO).

Drug Stability and Metabolism Studies

Liposomal and free CPT-11 were administered i.v. at a dose of 25 mg/kg in female albino rats (180-220 g) as above, and blood samples were withdrawn at intervals up to 48 hours. The blood samples were mixed with ice-cold PBS containing 0.04% EDTA and quickly centrifuged. The plasma was assayed for CPT-11, SN-38, and their carboxylate forms by HPLC using a modification of the method of Warner and Burke (19). Briefly, samples were extracted with 400 μL of ice-cold methanol by vortexing and centrifugation at 14,100 × *g* for 5 minutes. The mobile phase consisted of 3% triethylammonium acetate pH 5.5 (solution A) and acetonitrile (solution B) delivered at 1.0 mL/min in a linear gradient of 20 volume % A to 50 volume % B in 14 minutes. The eluted products were detected by fluorescence with an excitation at 375 nm and emission at 500 nm. The retention times were 5.3 minutes (CPT-11 carboxylate), 6.8 minutes (SN-38 carboxylate), 9.3 minutes (CPT-11), and 11.0 minutes (SN-38).

Conversion of CPT-11 to SN-38 was assayed in macrophages isolated from the peritoneum of female NCR *nu/nu* mice and plated at a density of 150,000 cells per well in a 12-well plate. After 24 hours, nanoliposomal CPT-11 was added to macrophages at a concentration of 10 μg CPT-11/mL and incubated for 24 hours in RPMI 1640 with 10% FCS. At indicated times, the medium was removed and the cells washed twice with Hanks buffered saline. The cells were treated with 0.2 mL of 1% Triton X-100 at room temperature for 5 minutes and solubilized in 0.8 mL of 80 volume % methanol/20 volume % 0.1 mol/L H_3PO_4 with shaking for an additional 5 minutes. The cell debris was removed by centrifugation at 13,000 rpm for 10 minutes and the supernatant was assayed by HPLC as described above.

Acute Toxicity Studies

The maximum tolerated dose following single i.v. administration was evaluated in healthy female Swiss Webster mice following a protocol adapted from the protocol communicated by the National Cancer Institute (NCI) Developmental Therapeutics Program. Briefly, in the first range-seeking step, the drug was administered via the tail vein in groups of two mice, beginning with the dose of 60 mg/kg CPT-11 and continuing with the dose escalation factor of 1.8 until acute mortality or terminal morbidity (within 1 day postinjection) was observed in any animal. The second range-seeking step was similarly done using a dose escalation factor of 1.15 and starting with the highest dose at which no mortality or terminal morbidity was observed (the highest tolerated dose) in the first step. Finally, in a validation step, a group of five mice were injected at the highest tolerated dose achieved in the second step and followed for up to 11 days for signs of general health daily and body weight twice a week. If during the observation period there was no mortality, irreversible (terminal) morbidity, or weight loss in excess of 15% of the preinjection body weight, the administered dose was considered the acute single injection maximum tolerated dose.

Antitumor Efficacy Studies

BT474 tumor model. NCR *nu/nu* athymic female mice (4-6 weeks old; Taconic Farms, Germantown, NY) were s.c. implanted at the base of tail with 60-day sustained release 0.72-mg 17β-estradiol pellets (Innovative Research of America, Inc., Sarasota, FL). Two days later, 2×10^7 BT474 human breast cancer cells were implanted s.c. in the upper back area as a 0.1-mL suspension. Tumor growth was measured by caliper along the largest (length) and smallest (width) axes twice a week. Tumor volumes were calculated using the following formula (20): tumor volume = [(length) × $(\text{width})^2$] / 2. At day 13 posttumor implantation (mean tumor volume, 200 mm^3), animals were randomized to three treatment groups of 13 to 15 animals per group and treated via i.v. (tail vein) injection as described in the text. The study was continued until day 60, which also represented the duration of estrogen supplementation. Animals were weighed twice weekly. If tumors reached 20% of the mouse body weight, the animals were euthanized.

HT29 tumor model. NCR *nu/nu* athymic male mice (6-week-old, weight >16 g; Charles River, Wilmington, MA) were injected s.c. in the right flank with 0.1-mL suspensions containing 5×10^6 HT-29 human colon cancer cells. Eleven days later (mean tumor volume, 150-350 mm^3), mice were randomized to six treatment groups of 11 animals per group. Starting on day 13, the animals received four tail vein injections at intervals of 4 days of various treatments as described in the text.

Results

Preparation of nanoliposomal CPT-11. A proposed novel process using a polyalkylammonium salt of a polymeric (polyphosphate) or nonpolymeric (sucrose octasulfate) highly charged multivalent anion as intraliposomal trapping agents resulted in improvement of both the encapsulation efficiency and the *in vivo* stability of the liposome-encapsulated weakly basic, amphipathic drug CPT-11. The process may involve the formation of an intraliposomal drug-polyanion complex (Fig. 1). Sucrose octasulfate is a high-charge density molecule with one strongly acidic, negatively charged sulfate group per 1.5 carbon atoms. The triethylammonium component of the salt assists drug loading as well, ensuring the charge neutrality of the liposome interior by allowing the efflux of cations accompanying the influx of the drug and possibly by formation of a self-perpetuating pH gradient to provide a driving force for progressive drug accumulation (10).

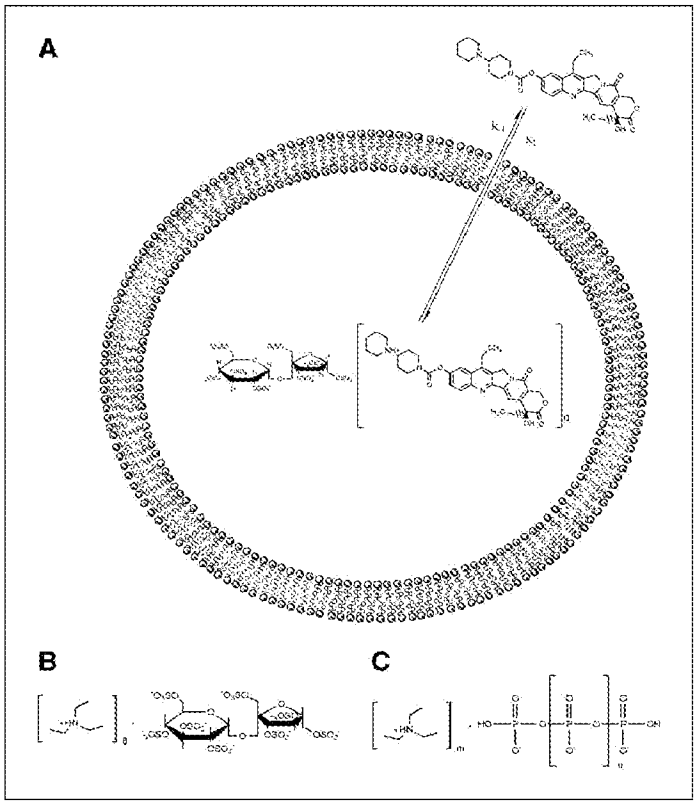

Figure 1. *A*, schematic depicting the intraliposomal stabilization strategy for CPT-11 using polyanionic trapping agents. The basic molecule CPT-11 forms a nanoscale complex with either poly(phosphate) or sucrose octasulfate in the liposome interior, thus stabilizing the liposomal formulation to increase drug retention while in circulation. Chemical structures of the triethylammonium salts of the polyanionic liposome trapping agents, poly(phosphate) (*B*) and sucrose octasulfate (*C*).

To minimize the treatment-associated lipid burden, encapsulation of CPT-11 was attempted up to drug-to-lipid ratios far exceeding the usual ratios achievable by traditional transmembrane-gradient drug loading techniques (Fig. 2). Remarkably, we found that CPT-11 encapsulation in liposomes was quantitative up to 800 g CPT-11/mol phospholipid. The final molar ratio of drug-to-phospholipid corresponds to 1.36:1 for liposomes loaded at 800 g CPT-11/mol phospholipid or 109,000 drug molecules per particle. This represents a 10- to 20-fold improvement over other liposomal formulations, including anthracyclines (3) or camptothecins lurtotecan (8) and SN-38 (21). We hypothesize that the high loading capacity of triethylammonium sucrose octasulfate liposomes is due to the formation of a stable complex between the drug and polyanion whereas the displaced triethylammonium ion dissociates and traverses the lipid bilayer as triethylamine, ensuring that the loading process continues until all added drug is encapsulated or the charge stoichiometry is achieved between the added drug and the liposomally encapsulated anion (Fig. 1).

Pharmacokinetics of nanoliposomal CPT-11. The pharmacokinetics of nanoliposomal CPT-11 formulated using either TEA-SOS or TEA-Pn were determined in normal female rats. Free CPT-11 was rapidly cleared from the circulation with $t_{1/2}$ = 0.27 hours (Fig. 3*A*). Liposome encapsulation was associated with significantly longer circulation times than free drug (Fig. 3*A* and *B*). This was especially true for liposomes loaded with TEA-SOS gradients, with blood half-lives for lipid and CPT-11 of 12.0 and 10.7 hours, respectively (Table 1).

Whereas both liposome constructs displayed long circulation for the lipid component, drug associated with TEA-SOS liposomes unexpectedly showed less rapid clearance from the blood than with TEA-Pn liposomes (Fig. 3*A* and *B*). This likely reflects that the $t_{1/2}$ of CPT-11 release from TEA-Pn liposomes was 14 hours, significantly shorter than that for TEA-SOS liposomes with a $t_{1/2}$ of CPT-11 release of 56.8 hours.

Drug stability of free and nanoliposomal CPT-11. *In vivo*, CPT-11 undergoes transformation to its more active metabolite, SN-38, and both molecules are also subject to inactivation by hydrolysis of the lactone forms to the respective carboxylate forms (Fig. 4*A* and *B*). Liposome encapsulation and delivery markedly altered these bioconversions in rats. Free CPT-11 was rapidly cleared from circulation, with only 2% of the injected dose remaining at 30 minutes and 35% of this present in the carboxylate form (Fig. 4*C*). In contrast, nanoliposomal CPT-11 showed both prolonged circulation, with 23.2% of injected dose still remaining at 24 hours, and drug protection, with no detectable conversion of CPT-11 to either SN-38 or the carboxylate form of CPT-11 (Fig. 4*D*). Thus, the high-charge density polyanionic nanoliposomal matrix provided a chaperone for the stably entrapped prodrug CPT-11, improving its pharmacokinetics and preventing its inactivation or premature conversion to the toxic metabolite SN-38.

Once deposited in tumors, liposomes are known to be taken up avidly by tumor-resident macrophages (22). To determine if macrophages could metabolically activate drug from nanoliposomal CPT-11, an *ex vivo* assay using macrophages isolated from the peritoneum of nude mice was done. Incubation of nanoliposomal CPT-11 with macrophages showed no detectable conversion to SN-38 at 24 hours but 100% conversion to SN-38 by 72 hours. This time course suggested that at least 24 hours was required for macrophage-mediated disruption of the liposome, drug release, and conversion to SN-38.

Acute toxicity of nanoliposomal CPT-11. The acute toxicity of free and nanoliposomal CPT-11 was determined in normal Swiss Webster mice using an NCI-based protocol. The maximum tolerated dose of free CPT-11 was 80 mg/kg whereas the maximum

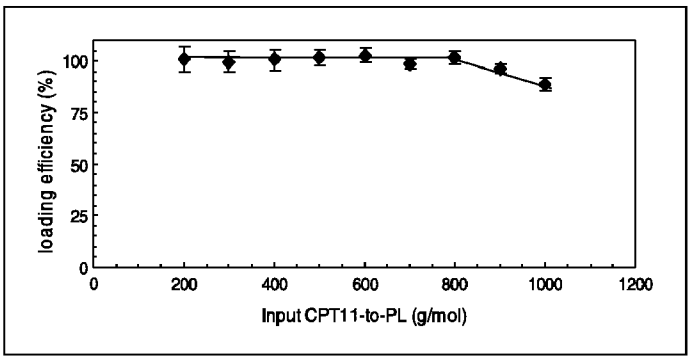

Figure 2. Liposomal loading efficiency as a function of input CPT-11-to-phospholipid (*PL*) ratio. Distearoylphosphatidylcholine/cholesterol/methoxypoly(ethylene)glycol (PEG2000)-derivatized distearoylphosphatidylethanolamine (3:2:0.015) liposomes were loaded with CPT-11 as described in Materials and Methods. The resulting CPT-11-to-phospholipid ratio following loading was determined by quantitating both CPT-11 and phospholipid in the resulting purified liposomal CPT-11 formulation, and the loading efficiency by comparing this ratio to the input ratio.

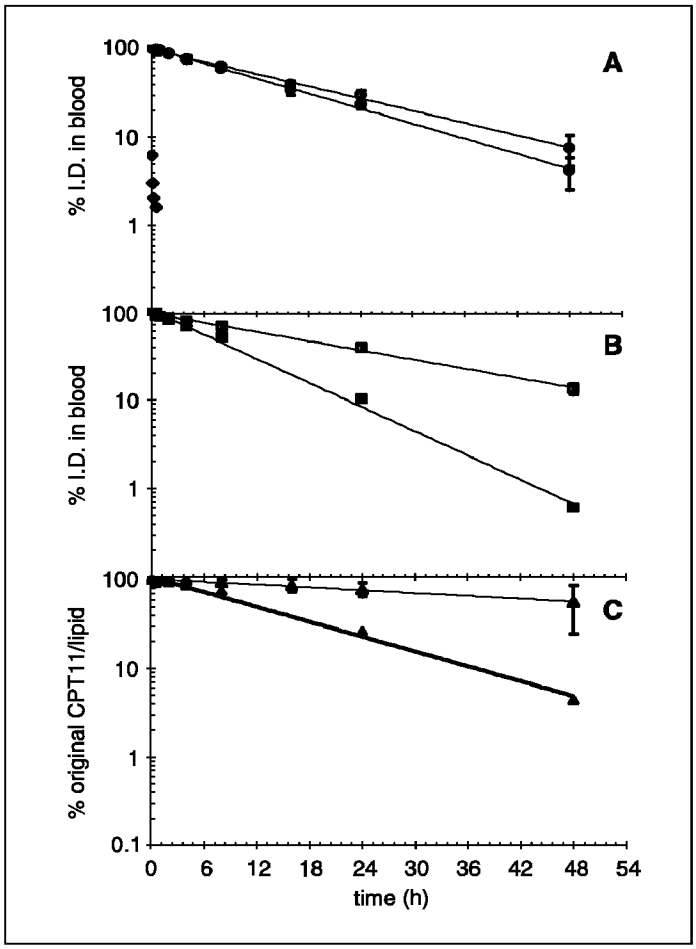

Figure 3. Pharmacokinetics of nanoliposomal CPT-11 in rats. Nanoliposomal CPT-11 prepared using either triethylammonium sucrose octasulfate (*A*) or poly(phosphate) (*B*) was administered i.v. in 9-week-old female Sprague-Dawley rats (body weight ~200 g) with indwelling central venous catheters at a dose of 10 mg CPT-11/kg (17.6 μmol phospholipid/kg). Free CPT-11 was administered i.v. as a bolus injection at 25 mg/kg (*A*, ◆). Plasma was sampled at the indicated times and analyzed for drug and liposomal lipid content. *Points*, % of injected dose (% *I.D.*) of lipid (○, □) or drug (●, ■, ◆). *C*, drug retention was calculated as percent of original drug associated with liposomal lipid at each time point for the poly(phosphate) (▲) and sucrose octasulfate (△) formulations.

tolerated dose of nanoliposomal CPT-11 formulated using a TEA-SOS gradient was not achieved even at the highest administered dose of 324 mg CPT-11/kg. A dose of >324 mg CPT-11/kg was impossible to administer because of concentration and injection volume limitations. Therefore, nanoliposomal CPT-11 delivery reduced drug toxicity in the mouse by at least 4-fold.

Efficacy of nanoliposomal CPT-11 in the BT474 breast cancer model. Treatment using nanoliposomal CPT-11, formulated using the TEA-Pn loading strategy, was evaluated in the BT474 breast tumor xenograft model (Fig. 5*A*). Free CPT-11 was clearly efficacious in this model with noticeable inhibition of tumor growth. However, treatment with nanoliposomal CPT-11 provided further advantage with dramatic regressions in tumor volumes and 100% cures of mice (defined as no residual tumor at study end).

Treatment-related toxicities were not observed. There was a slight decrease in mean body weight by 3.3% on the final treatment day in the animals receiving liposomal CPT-11; this decrease was not statistically significant compared with pretreatment weight (P = 0.274, Student's t test). All other weight measurements were within the expected range.

Efficacy of liposomal CPT-11 in the HT29 colon cancer model. In the HT29 colon tumor xenograft model, free CPT-11 again showed efficacy, albeit modest (Fig. 5*B*). However, both nanoliposomal CPT-11 formulations showed pronounced antitumor effects, including tumor regression during treatment followed by prolonged absence of tumor regrowth. Indeed, at 42 days postimplantation, all nanoliposomal CPT-11 treatments seemed to be equivalent and maximally efficacious.

With continued observation, tumor regrowth was observed beginning on day 47 postimplantation. At this point, all control and free CPT-11-treated mice had been sacrificed due to excessive tumor growth. Based on regrowth rates, treatment with TEA-SOS liposomes was more efficacious than TEA-Pn liposomes administered at the same CPT-11 dose. Furthermore, treatment with either liposome type at 50 mg/kg dose was more efficacious than at 25 mg/kg. In an analysis of cure rates, no mice receiving control or free CPT-11 were cured. Mice receiving TEA-Pn liposomal drug at 50 mg/kg per injection, despite initial tumor regressions, showed eventual regrowth. In the two groups receiving 25 mg/kg of either liposome formulation, one animal (9.1%) from each group was tumor-free at study end. In the group receiving 50 mg/kg of the TEA-SOS liposome formulation, 4 animals (36.4%) showed no regrowth and remained tumor-free.

Animals receiving free CPT-11, but not any of the nanoliposomal CPT-11 preparations, showed morbidity (loss of alertness, humped posture, ruffled fur, decreased mobility) for 1 hour after drug injection. Animals receiving free CPT-11 also lost 6% of weight

Table 1. Pharmacokinetic variables for free and nanoliposomal CPT-11 in rats

Formulation	$t_{1/2}$ (h)	AUC_∞ (μg h/mL)	CL (mL/h)	V_d (mL)	MRT (h)	$t_{1/2}$ CPT-11 release (h)
Free CPT-11	0.27	6.2	1,609	616.4	0.4	—
Ls-CPT-11 [TEA-Pn]	6.80	1,407.8	7.10	69.7	9.8	14.0
Ls-CPT-11 [TEA-SOS]	10.7	2,134.4	4.69	72.3	15.4	56.8

NOTE: The data used to calculate the pharmacokinetic variables for CPT-11 when formulated either in the free form or liposomal form refer to the actual drug concentrations measured in the blood that were then used to calculate the %ID values found in the corresponding curves for Fig. 3*B*.
Abbreviations: AUC_∞, area under the concentration versus time curve in plasma based on the sum of exponential terms; MRT, mean residence time calculated from exponential terms; CL, clearance calculated from exponential terms; V_d, volume of distribution.

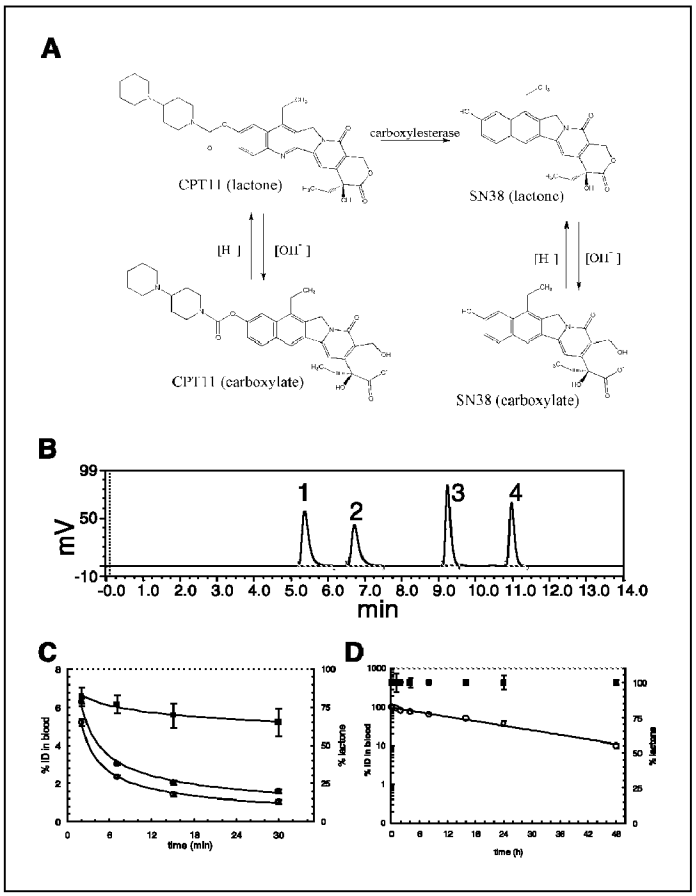

Figure 4. Drug stability of free and nanoliposomal CPT-11. *A*, CPT-11 and SN-38 exist in a pH-dependent equilibrium between closed lactone and open carboxylate configurations. CPT-11 is converted to its more active metabolite, SN-38, by carboxylesterases. *B*, HPLC chromatogram showing the separation of these species: CPT-11 carboxylate (*peak 1*), SN-38 carboxylate (*peak 2*), CPT-11 lactone (*peak 3*), and SN-38 lactone (*peak 4*). The *in vivo* drug stability of free (*C*) and nanoliposomal (*D*) CPT-11 was evaluated following single i.v. bolus administration at a dose of 25 mg CPT-11/kg in 9-week-old female Sprague-Dawley rats (body weight ~200 g). Levels of total CPT-11 (●) and CPT-11 lactone (○) in the blood were determined by HPLC analysis and expressed as percent of initial CPT-11 dose. *Right y axis*, percentage of CPT-11 in the lactone form is plotted as a function of time (■).

during treatment and did not recover, probably because of the effects of the growing tumor. Animals receiving nanoliposomal CPT-11 formulations experienced transient weight loss of 5% (at 25 mg/kg) or 9% (at 50 mg/kg) between the second and third injections as compared with pretreatment values; however, weights recovered following completion of treatment.

Discussion

Liposome delivery has been shown to improve the pharmacokinetic profile and widen the therapeutic index of certain anticancer drugs, especially the anthracycline class (1, 2). Improved efficacy is in part a result of passive targeting to tumor sites based on the enhanced permeability and retention (EPR) effect (23). To fully exploit this process, drug carriers must be engineered to retain drug while circulating, thereby preventing premature drug release before accumulating in the tumor but still allowing for release of drug once in the vicinity of the tumor. Antibody-targeted nanoparticles, such as immunoliposomes against HER2 (24) or epidermal growth factor receptor (25), represent another strategy for more efficient drug delivery to tumor cells.

Gradient-based drug loading technologies, in which electrochemical gradients drive the accumulation of drugs in the liposome interior, represent a key advance in liposome research (11, 26). This approach was further refined when transmembrane gradients of ammonium ion were proposed to form a self-sustaining pH-gradient that can load drugs inside liposomes (10). However, weakly basic anthracyclines represented the only drug class that afforded slow *in vivo* release rates when loaded using gradients involving common anionic counterions, such as sulfate or citrate. With other drug classes, gradient-based loading has been achieved with variable efficiency. To stabilize other cationic drugs against premature escape from liposomes, the use of pre-entrapped polyanionic polymers was proposed (9, 27).

In the present study, we used a drug loading transmembrane gradient system with two components, a substituted ammonium and a poly(anionic) trapping agent of either polymeric (polyphosphate) or nonpolymeric (sucrose octasulfate) nature. The use of polymeric polyanions such as heparin or dextran sulfate to improve liposomal drug retention has been reported (9, 27). Polyphosphate was effective in stabilizing intraliposomal CPT-11

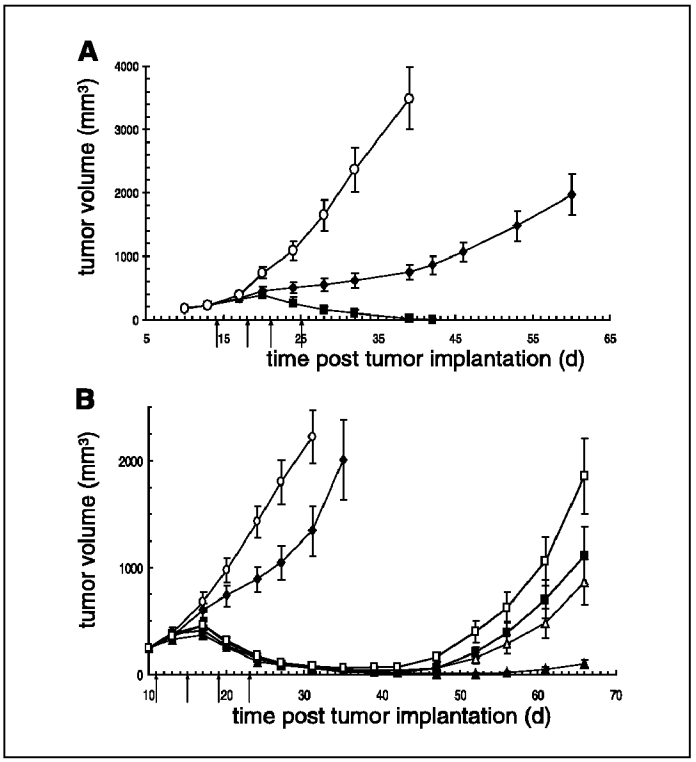

Figure 5. Antitumor efficacy of nanoliposomal CPT-11 in tumor xenograft models. *A*, BT474 breast cancer cells were implanted s.c. in nude mice along with estrogen pellets. When tumors were well established and had reached mean volumes of 200 mm³, the following treatments were initiated: control (○), drug- and liposome-free vehicle only; free CPT-11 (◆); or nanoliposomal CPT-11 stabilized with TEA-Pn (■). Free and nanoliposomal CPT-11 were injected at 50 mg CPT-11/kg/dose i.v. twice per week for four doses (*arrows*). *B*, HT-29 colon cancer cells were implanted s.c. in nude mice. When tumors were well established and had reached mean volumes of 150 to 350 mm³, the following treatments were administered: control (saline; ○); free CPT-11, 50 mg/kg/dose (◆); nanoliposomal CPT-11 using TEA-Pn, 25 mg/kg/dose (□); nanoliposomal CPT-11 using TEA-Pn, 50 mg/kg/dose (■); nanoliposomal CPT-11 using TEA-SOS, 25 mg/kg/dose (△); and nanoliposomal CPT-11 using TEA-SOS, 50 mg/kg/dose (▲).

against *in vivo* release, having the added advantage of being more readily biodegradable than dextran sulfate. However, polyanionic polymers such as heparin and dextran sulfate have notable anticoagulant activity and, in the case of dextran sulfate, toxic to Kupffer cells (28). The undefined chemical nature of many functionalized polymers may also contribute to variability in *in vivo* properties. Unexpectedly, we observed that a highly charged, nonpolymeric anion, such as sucrose octasulfate, provided even better drug retention than a polyanionic polymer, resulting in outstanding *in vivo* drug encapsulation stability. Sucrose octasulfate is a product of exhaustive esterification of sucrose, using chlorosulfonic acid or sulfur trioxide in pyridine or methylpyridine, and is a known pharmaceutical ingredient, the basic aluminum salt (Sucralfate) of which is widely used to treat gastric hyperacidity (29). Compared with dextran sulfate, sucrose octasulfate is chemically well defined; it does not have known anticoagulant or antimacrophage activity (29) and its salts can be produced in pure crystalline form ensuring less interlot variability.

The concept of nanoparticle delivery of camptothecins is very attractive based on potential advantages, including overcoming the solubility limitations of this class, protecting drug in the active lactone configuration, rerouting of drug from sites of toxicity such as the gastrointestinal tract, prolonging circulation time, increasing tumor accumulation via the EPR effect, and providing sustained release for a so-called metronomic effect. Using a novel intraliposomal stabilization technology, we have developed a nanoliposomal CPT-11 featuring drug loading efficiency and drug payload ($>10^5$ per particle) in far excess of that previously reported for this type of encapsulation; this agent showed marked *in vivo* retention of CPT-11 during long circulation times while simultaneously protecting the drug from lactone hydrolysis or premature activation. Compared with free CPT-11, this liposome-based nanoparticle reduced host toxicity in rats by >4-fold and greatly increased antitumor efficacy in animal models. In a separate study, we showed similar improvements in efficacy and host toxicity when nanoliposomal CPT-11 was administered locally to brain tumors using convection-enhanced delivery (30).

Previously reported liposomal camptothecin preparations have shown increased efficacy but not necessarily improved toxicity when compared with free drug (8, 9, 31). Other examples have shown prolonged circulation (32, 33), but not to the extent observed for the TEA-SOS-stabilized liposomes described here. In addition, a liposomal version of SN-38 is cleared even more rapidly with an AUC_∞ that seems to be at least 2 orders of magnitude less than that observed for nanoliposomal CPT-11 (34).

Another aspect of nanoliposomal CPT-11 is that it delivers a prodrug. Cytotoxic drugs encapsulated in liposomes are normally unable to act on their therapeutic targets or cause toxicity until they can be released from the confines of the carrier, and thus liposomal drug delivery can itself be regarded as a prodrug strategy. Hence, in this dual prodrug strategy, liposome delivery of CPT-11 chaperones the camptothecin until it reaches tumor sites where the prodrug can then be activated locally. Although local activation of CPT-11 to SN-38 has yet to be shown, carboxylesterases have a widespread distribution in different tumor types (35–37) and are active in macrophages, the principal scavenger of liposomes. Indeed, we observed that nanoliposomal CPT-11 was completely converted to SN-38 by macrophages after 72-hour incubation. We hypothesize that nanoliposomal CPT-11 may be acted on by tumor-resident macrophages, which convert drug to SN-38 with subsequent diffusion to nearby tumor cells. Alternatively, CPT-11 may be activated directly by tumor cells following release from its liposome carrier.

We conclude that nanoliposomal CPT-11 generated by novel intraliposomal drug stabilization resulted in advantageous pharmacologic properties with increased efficacy and reduced host toxicity *in vivo*. The drug-loading and stabilization technologies used for CPT-11 may also be broadly applicable to other weakly basic anticancer drugs as we have recently shown using a novel histone deacetylase inhibitor, LAQ824 (38). Nanoliposomal CPT-11 may provide a robust and useful nanoparticle-based treatment for cancer.

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Optimizing Liposomes for Delivery of Chemotherapeutic Agents to Solid Tumors

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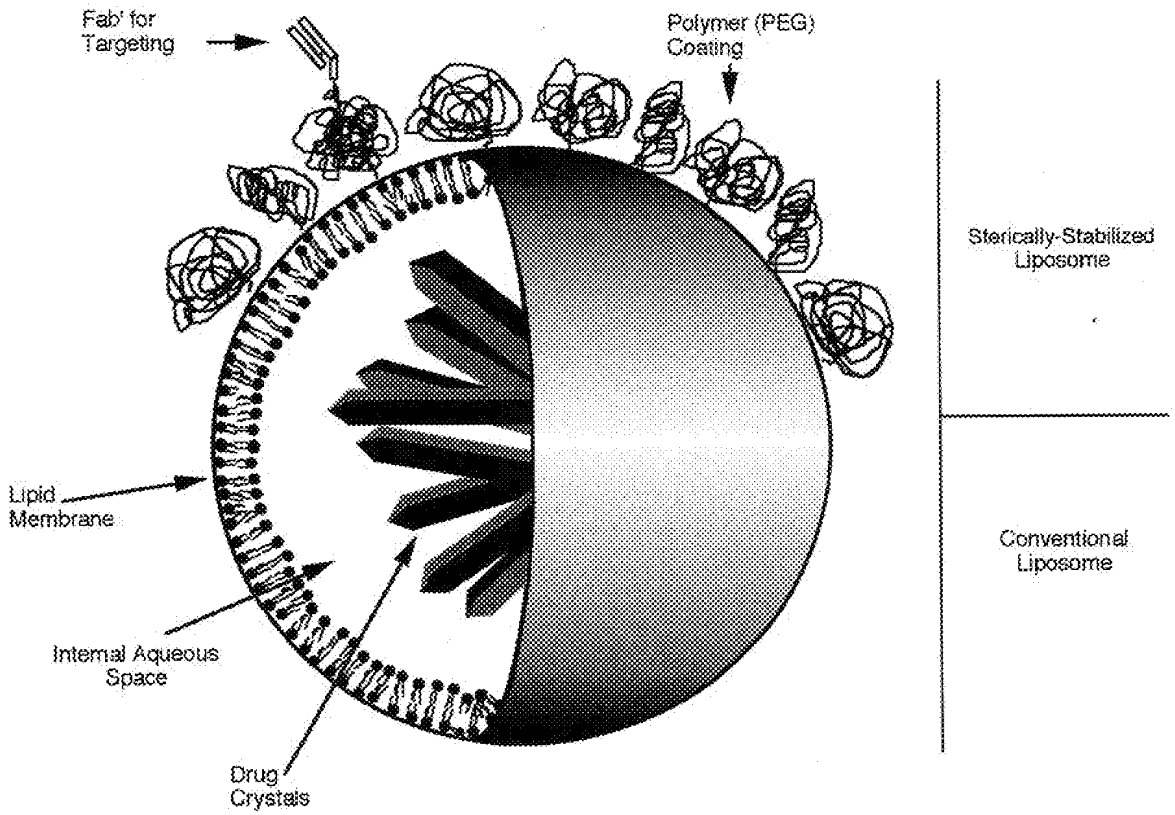
I. Introduction

There are many potential barriers to the effective delivery of a drug in its active form to solid tumors. Most small-molecule chemotherapeutic agents have a large volume of distribution on i.v. administration (Speth et al., 1988; Chabner and Longo, 1996). The result of this is often a narrow therapeutic index due to a high level of toxicity in healthy tissues. Through encapsulation of drugs in a macromolecular carrier, such as a liposome, the volume of distribution is significantly reduced and the concentration of drug in the tumor is increased (see *IIF. Comparison of Pharmacokinetic Parameters for Different Liposomal Formulations* and *III. Accumulation of Liposomal Drugs in Tumors*). This results in a decrease in the amount and types of nonspecific toxicities and an increase in the amount of drug that can be effectively delivered to the tumor (Papahadjopoulos and Gabizon, 1995; Gabizon and Martin, 1997; Martin, 1998). Under optimal conditions, the drug is carried within the liposomal aqueous space while in the circulation but leaks at a sufficient rate to become bioavailable on arrival at the tumor. The liposome protects the drug from metabolism and inactivation in the plasma, and due to size limitations in the transport of large molecules or carriers across healthy endothelium, the drug accumulates to a reduced extent in healthy tissues (Mayer et al., 1989; Working et al., 1994). However, discontinuities in the endothelium of the tumor vasculature have been shown to result in an increased extravasation of large carriers and, in combination with an impaired lymphatics, an increased accumulation of liposomal drug at the tumor (see *III. Accumulation of Liposomal Drugs in Tumors*; Huang et al., 1993; Yuan et al., 1994, 1995; Hobbs et al., 1998). All of these factors have contributed to the increased therapeutic index observed with liposomal formulations of some chemotherapeutic agents (Papahadjopoulos et al., 1991; Gabizon, 1994; Martin, 1998).

A diagram depicting both a conventional liposome (CL)[3] and a sterically stabilized liposome (SSL) is shown in Fig. 1. The two types of liposomes share a lipid membrane that is relatively impermeable to both amphipathic and highly water-soluble molecules at physiological temperatures (37°C). This feature is important for the maintenance of stable liposome drug formulations, both during storage and in plasma (see *VII. Stability in Plasma and Storage*). Liposomes composed of a comparably more-fluid membrane are being used as a rapid-

[3] CL, conventional liposome; SSL, sterically stabilized liposome; ara-C, 1-β-D-arabinofuranosylcytosine; AUC, area under the curve for concentration versus time; ABV, doxorubicin/bleomycin/vincristine; BV, bleomycin/vincristine; Chol, cholesterol; DOX, doxorubicin; DPPC, 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine; DPPE, 1,2-dipalmitoyl-3-*sn*-phosphatidylethanolamine; DPPG, 1,2-dipalmitoyl-3-*sn*-phosphatidylglycerol; DSPA, 1,2-dipalmitoyl-3-*sn*-phosphatidic acid; DSPC, 1,2-distearoyl-3-*sn*-phosphatidylcholine; DSPG, 1,2-distearoyl-3-*sn*-phosphatidylglycerol; eggPC, phosphatidylcholine derived from egg yolk; H-F, hand and foot; HSPC, hydrogenated soy phosphatidylcholine; ILS, increased life span; L-DOX, liposomal doxorubicin; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEG, polyethylene glycol; PEG-DSPE, *N*-(polyethylene glycol)distearoylphosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; RES, reticuloendothelial system; SSL DOX, sterically stabilized liposomal doxorubicin; VCR, vincristine; CSF, colony-stimulating factor; POPC, 1-palmitoyl, 2-oleoyl-3-*sn*-phosphatidylcholine.



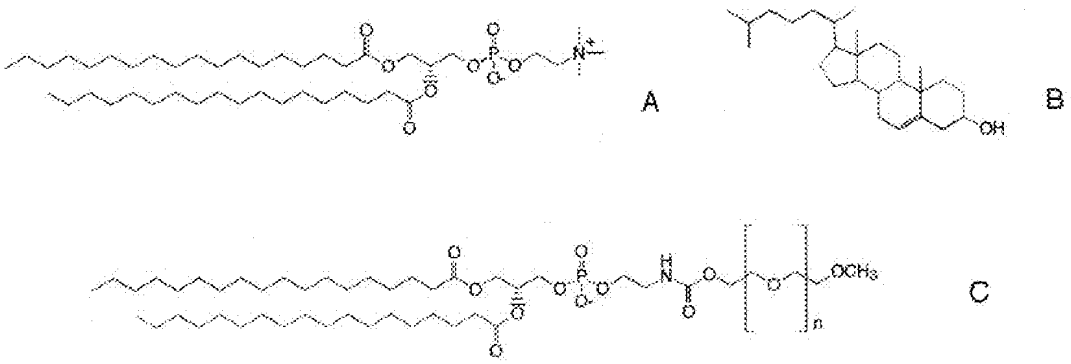


FIG. 1. Diagram of a drug-loaded liposome both with (SSL) and without (CL) a PEG coating. The liposome contains a lipid membrane that encapsulates an internal aqueous space used to entrap chemotherapeutic drugs. DOX can be encapsulated at concentrations exceeding its aqueous solubility, forming drug crystals in the liposome interior. Alternatively, some drugs can be carried within the lipid bilayer. Further modifications of the surface through covalent attachment of targeting ligands such as Fab' fragments can result in liposomes that are specifically endocytosed by cancer cells expressing a receptor for that ligand (e.g., the HER2 receptor found on certain breast cancer tumors). The structures of the three most commonly used lipids that compose the lipid bilayer are also given. DSPC (A) or an equivalent, HSPC, is the primary phospholipid component, whereas Chol (B) is the neutral lipid component. PEG-DSPE (C) is incorporated at concentrations of 4 to 6 mol% in SSL formulations.

release system for doxorubicin (DOX) and are described to a limited extent in this review. A liposome also has an internal aqueous space, which can be used to entrap a variety of chemotherapeutic drugs or diagnostic dyes. We discuss in *VII. Stability in Plasma and Storage* how different drugs are efficiently loaded into this space. The

two types of liposomes differ in the presence of the polymer coating [most commonly, polyethylene glycol (PEG)] on the surface of the SSLs but not CLs. This coating provides steric stabilization to the liposome, which is thought to limit binding of serum opsonins as well as direct interactions with cells, most importantly, of the reticuloendothelial system (RES; Allen et al., 1991, 1994; Lasic et al., 1991). The result is enhanced circulation times and increased localization in the tumor (Papahadjopoulos et al., 1991, 1995; Gabizon and Martin, 1997).

Steric stabilization refers to the colloidal stability (Lasic and Needham, 1995; Lasic and Papahadjopoulos, 1996) conferred on the liposome by a variety of hydrophilic polymers or hydrophilic glycolipids (Allen and Chonn, 1987; Papahadjopoulos et al., 1991; Woodle and Lasic, 1992; Allen, 1994; Torchilin et al., 1995; Zalipsky et al., 1996), the best studied of which are PEG and the ganglioside GM_1. An important finding was that SSLs also show a prolonged lifetime in the circulation (Allen and Chonn, 1987; Gabizon and Papahadjopoulos, 1988; Klibanov et al., 1990; Allen et al., 1991; Papahadjopoulos et al., 1991). SSLs then typically refer to any liposomes containing PEG-PE, GM_1, or another of these glycolipids or polymers that has a relatively long half-life in the general circulation. The term "conventional liposomes" has a much broader definition and refers to liposomes composed of a variety of different lipid compositions, but typically the most commonly used of these compositions are very high in phosphatidylcholine (PC) and cholesterol (Chol). The pharmacokinetics and tissue distribution of CLs depend on properties such as size, surface charge, and membrane packing. These factors are discussed in more detail in *II. Pharmacokinetics and Biodistribution of Liposomes and Liposomal Drugs*. However, to perform a careful comparison, we limit this discussion to formulations optimized for increased residence in the circulation, accumulation in tumors, and stability in the plasma. For SSLs, we consider liposomes containing 4 to 6 mol% PEG-DSPE, ~30 mol% Chol, and the remainder hydrogenated soy phosphatidylcholine (HSPC) or distearoylphosphatidylcholine (DSPC; Fig. 1). The size of the carrier is usually 60 to 120 nm. For CLs, the optimized formulations are composed of DSPC and Chol in either a 55:45 or 66:33 M ratio or phosphatidylcholine derived from egg yolk (eggPC)/Chol (3:2) and have a similar average size distribution.

The choice of drug for delivery via liposomes is essential to the success of this approach. Broad generalizations as to the usefulness of a certain liposome composition for the delivery of all chemotherapeutic drugs or as to the superiority of liposomal formulation for all classes of drugs is extremely dangerous considering the present limitations in liposome technology. To be effective as a carrier, a liposome must be able to efficiently balance stability in the circulation with the ability to make the drug bioavailable at the tumor. In choosing a drug, there are several criteria to consider. The drug must have sufficient activity against the chosen tumor; a drug such as DOX with a relatively broad activity against a variety of different tumor models is an ideal choice in this regard (Young et al., 1981; Doroshaw, 1996). Second, the drug must be efficiently loaded into the liposomal carrier. Ammonium sulfate and pH gradients have been used for remote loading of a variety of amphipathic basic amines, resulting in encapsulation efficiencies of ~100% (Madden et al., 1990; Lasic et al., 1992a; Haran et al., 1993; Cullis et al., 1997). Finally, the drug must be compatible with the carrier; it must be stably transported in the circulation but still released at the tumor. A wide array of different drugs have been encapsulated in liposomes for the treatment of cancer (Fig. 2; Heath et al., 1983; Papahadjopoulos et al., 1991; Allen et al., 1992; Vaage et al., 1993b; Burke and Gao, 1994; Sharma et al., 1995; Jones et al., 1997; Working, 1998). The listed examples illustrate a diversity of different classes of chemotherapeutic drugs, with distinct chemical stabilities, solubility and membrane partitioning properties, modes of action, and modes of drug resistance.

Barenholz and coworkers (Barenholz and Cohen, 1995; Barenholz, 1998) classified these drugs into one of three classes depending on their hydrophobic properties measured as octanol-to-water partition coefficient (K_p): 1) highly hydrophilic drugs such as *N*-(phosphonoacetyl)-L-aspartate, 2) hydrophobic drugs such as paclitaxel, and 3) amphipathic drugs such as DOX, which represent many current chemotherapeutic agents. Liposomal formulations of highly hydrophilic drugs can be limited by the bioavailability of these drugs at the tumor site, which may be prohibitively low due to their extremely low membrane permeability and, therefore, low drug release once the carrier has reached the tumor. Drugs such as 1-β-D-arabinofuranosylcytosine (ara-C) or methotrexate, which are taken by tumor cells using membrane transporters (Plageman et al., 1978; Wiley et al., 1982; Westerhof et al., 1991, 1995; Antony, 1992), may be useful members of this class of drugs, assuming they can be released from the liposome in adequate quantities (Heath et al., 1983; Matthay et al., 1989; Allen et al., 1992). Future improvements in the design of carriers that are destabilized and release the drugs specifically at the tumor site may make their utilization more feasible, as discussed in *VIII. Bioavailability of Encapsulated Drug*. Highly hydrophobic drugs tend to associate mainly with the bilayer compartment of the liposome; this leads to lower entrapment stability due to faster redistribution of the drug to plasma components. However, liposomes may be used with this class of drugs simply as the means to formulate them for i.v. administration rather than using liposome encapsulation to achieve enhanced tumor delivery of the drugs. For example, paclitaxel has formulated into liposomes (Sharma et al., 1995, 1997) but may be equally suitable when formulated as a microemulsion (Wheeler et al., 1994). Liposomes have also used to solubilize and

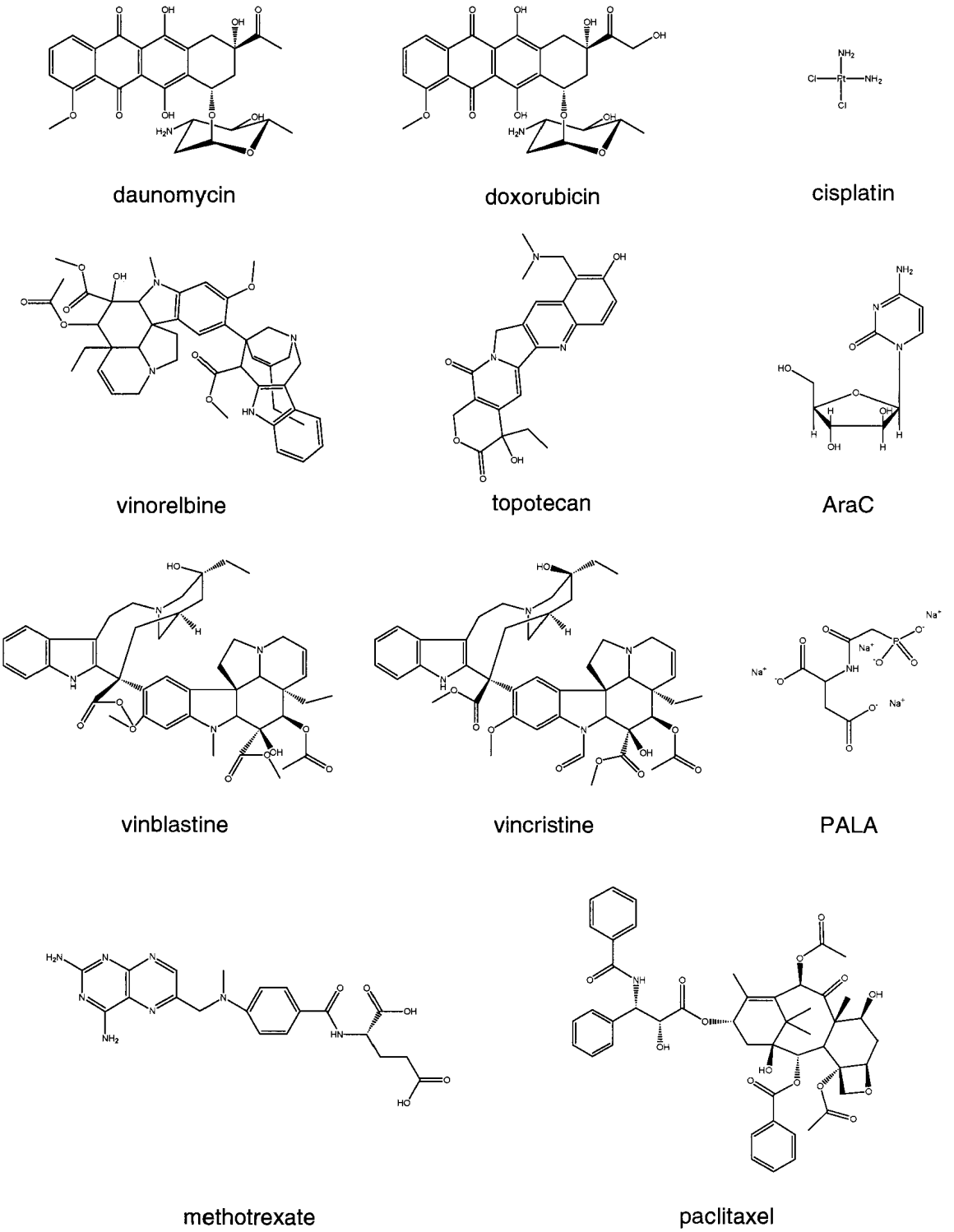


FIG. 2. Structures of a few chemotherapeutic drugs that have been used with liposomes either in vitro or in vivo. These drugs operate via a variety of different mechanisms; some have different mechanisms of drug resistance and varying physical characteristics that make them more or less compatible for encapsulation in liposomes. At the present time, only formulations of anthracyclines (daunorubicin and DOX) have been sufficiently developed for use in the clinic, although a liposomal VCR formulation is presently under study in clinical trials.

administer hydrophobic photosensitizers for use in photodynamic therapy (Allison et al., 1990; Reddi, 1997).

Considering the present state of liposome technology, amphipathic drugs appear to be the most suitable for liposomal carriers; these drugs include anthracyclines, such as DOX and daunorubicin, and *Vinca* alkaloids, such as vincristine (VCR), vinblastine, and vinorelbine (Fig. 2). With this class of drugs, it is possible to tune the drug-release rates to maintain the stability of the formulation in the plasma, yet allow the drug to be released at the tumor site. This is in large part due to the development of gradient-based loading techniques leading to

stable liposomal drug formulations (Nichols and Deamer, 1976; Mayer et al., 1985; Madden et al., 1990; Haran et al., 1993). Indeed, the first liposomal oncology drugs approved for medical use in liposomal form are of the anthracyclines daunorubicin (DaunoXome; Nexstar Pharmaceuticals, Boulder, CO) and DOX [Doxil; Alza Corporation, Palo Alto, CA (CAELYX in Europe)]. DaunoXome is formulated as a CL (DSPC/Chol), whereas Doxil is an SSL formulation (hydrogenated soy PC/Chol/PEG-DSPE; Table 1). Another CL formulation (eggPC/Chol) of DOX (Harris et al., 1998), as well as formulations of other amphipathic drugs, such as VCR (Embree et al., 1998) or cisplatin (Newman et al., 1999), is in preclinical or clinical trials or under Food and Drug Administration consideration for commercial release.

In the remaining sections of this review, we attempt to show how optimization of the balance of circulation lifetimes, drug-induced toxicities, accumulation in tumors, and drug release rates from liposomes results in the most clinically effective formulations. This is accomplished through adjustments of both the pharmacological and physical properties of the liposome, including the injected dose, liposome size, presence of steric stabilization, and lipid composition of the carrier.

II. Pharmacokinetics and Biodistribution of Liposomes and Liposomal Drug

For free DOX, the volume of distribution has been estimated at 25 l/kg, suggesting a significant uptake by tissues (Speth et al., 1988). This large volume of distribution, when combined with the relatively rapid clearance rate from the circulation, results in low drug levels in the tumor and significant toxicity to normal tissues. Liposomes can alter both the tissue distribution and the rate of clearance of a drug by causing the drug to take on the pharmacokinetic parameters of the carrier. Pharmacokinetic parameters of the liposomes depend on physicochemical attributes of the liposomes, such as size, surface charge, membrane lipid packing, and steric stabilization, as well as on the administered dose and route of administration. The pharmacokinetics of both CLs and SSLs have been extensively reviewed (Hwang, 1987; Allen et al., 1995; Allen and Stuart, 1999).

Both slow-release CLs and SSLs have a volume of distribution for DOX not significantly different from the total blood volume (see Table 3), indicating the drug is generally confined to the systemic circulation. However, after i.v. administration, CLs have saturable, nonlinear kinetics, whereas SSLs have nonsaturable, log-linear kinetics (Hwang, 1987; Allen et al., 1995). The dose-dependent kinetics for CLs result in relatively rapid clearance rates for liposomes at low doses and complicates the calculations of clinical dosages. Clearance of CLs has been suggested by Allen et al. (1995a) to be due to both a high-affinity, low-capacity system, likely the macrophages of the RES, and a low-affinity, high-capacity system. Steric stabilization slows uptake by the high-affinity, low-capacity system, resulting in dose-independent kinetics. The potential mechanisms responsible for the reduced clearance and dose-independent pharmacokinetics of SSLs are described in more detail in *IIE. Effect of Steric Stabilization on Pharmacokinetic Parameters.*

Liposomes are cleared from the circulation by macrophages of the RES, in particular those of the liver and spleen (Gregoriadis, 1976; Weinstein, 1984; Senior, 1987). Opsonization by serum proteins such as the complement C3b fragment, β_2-glycoprotein I, and the Fc portion of IgG molecules is thought to play a critical role in the recognition and subsequent clearance by RES macrophages (Senior, 1987; Patel, 1992; Devine et al., 1994; Chonn et al., 1995; Devine and Marjan, 1997). The success of a liposome-based approach for drug delivery to sites other than those making up the RES is one that limits the uptake of liposomes by macrophages, either directly by preventing the interaction of liposomes with receptors on the macrophage surface or indirectly by decreasing the binding of serum opsonins. Many studies have concentrated on understanding the mechanisms responsible for regulation of these interactions. These factors are often intricately intertwined, making it impossible to construct sweeping assumptions based on any one factor.

A. Effect of Liposome Size on Pharmacokinetic Parameters

The first aspect of a liposome that affects its disposition is size. Liposomes of a defined size are readily prepared by extrusion of lipid suspensions through filters containing pores of a similar size (Olson et al., 1979; Szoka et al., 1980). Liposomes prepared through this method are slightly larger (20–50%) than the average pore size of the filter. The general trend for liposomes of similar composition is that increasing size translates into more rapid up-

TABLE 1
Commercial liposome formulations of anthracyclines

Drug	Manufacturer	Active Ingredient	Size	Lipid Composition	Drug/Lipid
			nm		*w/w*
Doxil (CAELYX)[a]	Alza Corporation[b]	DOX	100	HSPC/Chol/PEG-DSPE (56:39:5)	0.125:1
DaunoXome	Nexstar Pharmaceuticals	Daunorubicin	45	DSPC/Chol (2:1)	0.079:1
EVACET (TLC D-99)[c]	The Liposome Company, Inc.	DOX	150	eggPC/chol (55:45)	0.250:1

[a] Doxil (DOX) is known as CAELYX in Europe.
[b] Originally developed by Sequus Pharmaceuticals, Inc.
[c] EVACET was previously known as TLC D-99.

take by the RES (Abra and Hunt, 1981; Hwang, 1987; Senior, 1987). However, although the trend remains the same, the clearance of liposomes is affected to differing extents depending on the composition. For example, DSPC/Chol (3:2) liposomes extruded through 400-nm filters are cleared 7.5 times as fast as liposomes extruded through 200-nm filters, which in turn are cleared 5 times as fast as small unilamellar vesicles (Senior et al., 1985). The inclusion of PEG-DSPE in the liposome composition results in clearance rates that are relatively insensitive to size in the range of 80 to 250 nm (Allen et al., 1989; Liu et al., 1992; Woodle et al., 1992). Now, a 2-fold increase in size from 100 to 200 nm results in only a 54% increase in clearance (Fig. 3; Woodle et al., 1992). A similar dependence of liposome clearance on size was observed for DSPC liposomes stabilized with small quantities of *N*-glutaryl-phosphatidylethanolamines (Ahl et al., 1997). These liposomes also showed an increased plasma area under the curve (AUC) compared with DSPC/Chol controls, similar to PEG-DSPE-stabilized liposomes. The authors suggested that the aggregation of nonstabilized neutral liposomes may result in an increase in the effective size and, thus, clearance from the circulation via a size-dependent mechanism. Although the dependence of liposome clearance rates on size is relatively less for these two stabilized formulations than for that with CLs, it nevertheless highlights the importance of optimization of liposome size in drug delivery systems not aimed at the RES. For neutral CLs, the window for optimal behavior is considerably narrower, and these data suggest that liposomes should be small enough (preferably <100 nm) but still maintain reasonable drug encapsulation efficiencies.

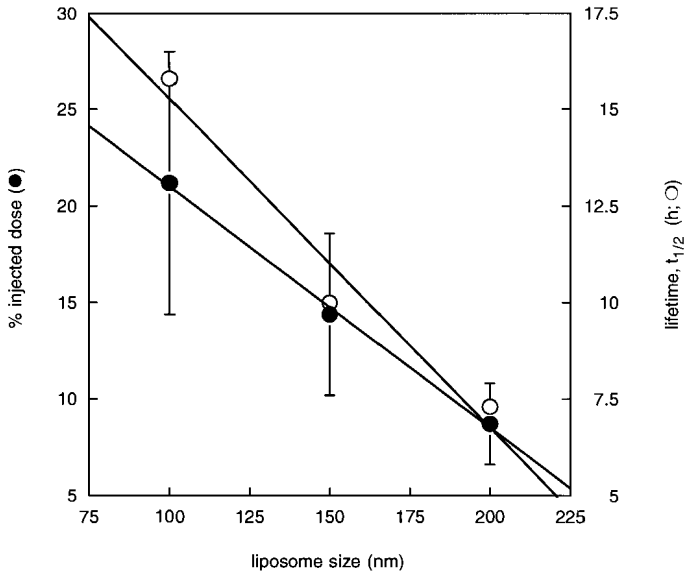


FIG. 3. Effect of liposome size on plasma levels of PEG-DSPE/PC/Chol (0.15:1.85:1 mol/mol/mol). Female adult Sprague-Dawley rats were injected with ^{67}Ga-desferoxamine-loaded liposomes, and blood was drawn at prescribed time points, when ^{67}Ga levels were determined with a gamma counter. From these data, the half-life in the circulation was determined by fitting the data to a single exponential curve (○), and the 24-h time point was recorded (●). The liposomes were prepared by extrusion through polycarbonate filters of defined size as described by Olson et al. (1979), and their size distribution was determined by dynamic light scattering. PC in these liposomes refers to partially hydrogenated egg PC. This figure was adapted from Woodle et al. (1992).

B. Effect of Lipid Dose on Pharmacokinetic Parameters

The administered dose can also play a significant role in the circulation lifetime of a carrier. CLs are removed from the circulation in a dose-dependent manner, indicating a saturation of the mechanisms responsible for their uptake (Gregoriadis and Senior, 1980; Abra and Hunt, 1981; Senior et al., 1985; Hwang, 1987). Circulation lifetimes typically increase as a function of increasing lipid dose. This effect is likely due to a decreased phagocytic capacity of RES macrophages after the ingestion of high lipid doses or to a saturation of plasma factors that bind to circulating liposomes and result in their opsonization. The fact that liposomes composed of high-phase transition lipids, such as SM/Chol or DSPC/Chol, can more readily saturate RES uptake may indicate that these difficult-to-metabolize lipids saturate metabolic pathways responsible for their destruction (Senior et al., 1985; Hwang, 1987). Alternatively, liposomes have been shown to bind serum proteins in a manner inversely proportional to their blood clearance rates (Chonn et al., 1992; Semple and Chonn, 1996; Semple et al., 1996), giving rise to the hypothesis that the depletion of plasma opsonins at high lipid doses results in an increase in blood circulation half-lives ($T_{1/2}$; Harashima et al., 1993; Oja et al., 1996).

RES blockade can also be achieved by delivering cytotoxic drugs such as DOX or dichloromethylene diphosphonate to RES macrophages (Bally et al., 1990b; Parr et al., 1993; Qian et al., 1994; Buiting et al., 1996). Parr et al. (1997) recently considered the effect of dose on DOX-loaded liposomes. In these experiments, the presence of DOX resulted in a ~1.5- to 2-fold increase in the plasma levels of liposomal lipid at higher doses for SSL DOX compared to CL DOX. In DOX-loaded liposomes, a 10-fold increase in plasma levels of liposomal lipid observed at lower lipid doses (<1 μmol lipid/20–22 g mouse) was reduced to a 3-fold increase at higher doses (>2 μmol lipid/mouse). It should be noted it is at these lower doses that SSL preparations are routinely used. Thus, with SSL DOX, long circulation does not necessarily come at the expense of RES toxicity. The possible implications of the use of dose escalation, and the resulting RES toxicity, simply to achieve long circulation times are described in more detail in *VI. Toxicology of Liposomal Chemotherapy*. This indicates that RES blockade is in part due to the drug and not solely to a saturation of plasma opsonins or inability to metabolize liposomal lipid components.

Steric stabilization with PEG-DSPE offers a unique advantage to liposome delivery in that clearance kinetics become dose independent (Allen and Hansen, 1991; Huang et al., 1992; Woodle et al., 1992). The data in Fig. 4 illustrate the relative effect of liposome dose on

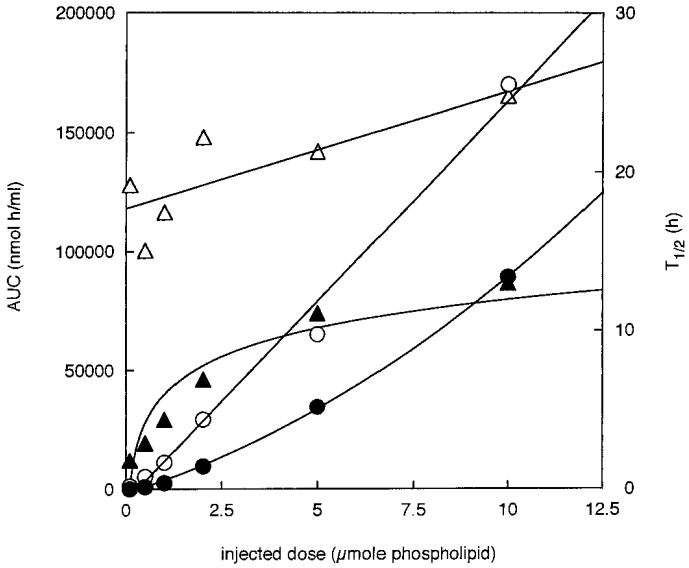


FIG. 4. Dependence of circulation $T_{1/2}$ values (▲, △) and plasma AUCs (●, ○) on administered dose. Female ICR mice (three per group) were given a single bolus tail-vein injection of liposomes containing ^{125}I-tyraminylinulin and 0.1 to 10 μmol of phospholipid. Liposomes were composed of either eggPC/Chol (2:1 mol/mol) or SM/eggPC/Chol/PEG-DSPE (1:1:1:0.2 mol/mol/mol). This figure was adapted from Allen and Hansen (1991).

clearance of both an SSL formulation (SM/eggPC/Chol/PEG-DSPE, 1:1:1:0.2) and a CL formulation (eggPC/Chol, 2:1). For the SSLs, the plasma AUC increases linearly, whereas the $T_{1/2}$ remains relatively unchanged. This dose independence was recently shown to extend down to concentrations of lipid as low as 1 μmol/kg in rabbits (Utkhede and Tilcock, 1998). In stark contrast, the plasma AUC for CLs increases slowly at low doses (<2.5 μmol phospholipid/23–27 g mouse) and then increases exponentially with increasing lipid dose. A look at the circulation $T_{1/2}$ of the conventional formulation shows a leveling off of the $T_{1/2}$, indicating a saturation of the mechanism responsible for their clearance. Although the CLs used in this particular study used a fluid-phase phospholipid component, eggPC, similar pharmacokinetics have been seen with DSPC/Chol and SM/Chol liposomes (Beaumier et al., 1983; Hwang, 1987; Chow et al., 1989). In one of these studies (Beaumier et al., 1983), liposome levels in the liver were shown to saturate at the same dose where plasma clearance rates leveled off, consistent with RES saturation being responsible for increased plasma levels at high lipid doses.

C. Effect of Liposome Charge on Pharmacokinetic Parameters

The effect of liposome surface charge on liposome clearance kinetics is an increasingly misused predictive factor of circulation lifetimes. Early studies have shown that the presence of negatively charged lipids in liposomes, including phosphatidic acid (PA), phosphatidylserine (PS), and phosphatidylglycerol (PG), results in rapid uptake by the RES (Senior et al., 1985; Senior, 1987). However, this relationship between the presence of charged lipids and circulation lifetimes is extremely complex and cannot be readily explained with simple models in which the presence of an anionic lipid necessitates increased clearance from the circulation. Indeed, it now appears that each lipid must be analyzed separately and in the context of similar liposomes with respect to size, membrane packing constraints, and surface charge density.

A more careful characterization of the effect of surface charge on liposome clearance in mice was conducted using liposomes containing different anionic phospholipids (Gabizon and Papahadjopoulos, 1992). In these experiments, anionic lipids were added to fluid eggPC/Chol liposomes in a 1:10:5 ratio (anionic lipid/eggPC/Chol). Although liposomes containing PG, PA, and PS (PS > PA > PG) were cleared more rapidly than neutral liposomes, the inclusion of other anionic lipids such as the ganglioside GM_1 or phosphatidylinositol (PI) resulted in longer circulation lifetimes. Later, this second group of anionic lipids was shown to include PEG-PE conjugates (Papahadjopoulos et al., 1991; Woodle et al., 1992). The new model then divided negatively charged lipids into those with and those without a sterically shielded negative charge. Those with a sterically hindered charge were cleared more slowly, whereas those without were cleared more rapidly than neutral liposomes of a similar composition. This too may have proved to be too simple of a model.

The last statement concerning a similar composition is extremely important, and the effect of the phase transition of the lipid is intricately interrelated with the effect of charge. 1,2-dipalmitoyl-3-*sn*-phosphatidylglycerol (DPPG)/DSPC/Chol liposomes were previously shown to be cleared more rapidly than DSPC/Chol liposomes in mice (Fig. 5; Lasic et al., 1991; Woodle et al., 1992), and in a separate study, eggPG/DSPC/Chol liposomes were cleared more rapidly than DPPG/DSPC/Chol liposomes (Gabizon et al., 1990). However, DSPC has a gel-to-liquid crystalline phase transition (T_m) of 55°C, whereas the T_m value of DPPG is 41.1°C (Table 2; Boggs et al., 1989). Thus, the replacement of some of the DSPC with DPPG does not necessarily result in liposomes with similar permeability and membrane packing characteristics. Recently, DOX-loaded 1,2-distearoyl-3-*sn*-phosphatidylglycerol (DSPG)/HSPC/Chol liposomes, in which the source of PG was distearoylphosphatidylglycerol (T_m = 53.0; Table 2), were shown to have plasma levels of DOX at 24 h that were greater than twice those of HSPC/Chol liposomes (Gabizon et al., 1996). In addition, the requirement for a high phase transition anionic lipid component may also be necessary for PI, where hydrogenated soy PI is most commonly used as the source of PI in long-circulating liposomes (Gabizon and Papahadjopoulos, 1988; Gabizon et al., 1990). Thus, from these few cases, it appears that that the dependence of long circulation is more related to

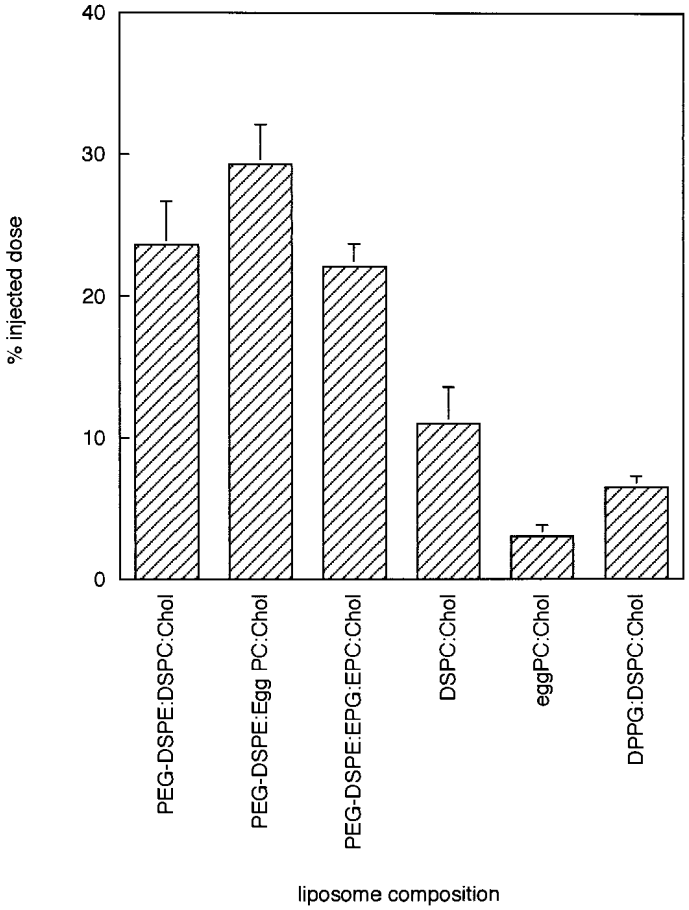


FIG. 5. Effect of steric stabilization and lipid composition on plasma levels of liposomes. Extruded liposomes (70–100 nm) loaded with ^{67}Ga-deferoxamine were injected by i.v. administration into female Swiss–Webster mice at a dose of 1 μmol of phospholipid per mouse. Blood levels of ^{67}Ga were determined by gamma counting 24 h after injection. Lipid molar ratios were 1:10:5 except for eggPC/Chol and DSPC/Chol, both at 10:5, and PEG-DSPE/EPG/eggPC/Chol at 1:3:7:5. This figure was adapted from Woodle et al. (1992) and Lasic et al. (1991).

TABLE 2
Primary gel-to-liquid crystalline phase transitions of different phospholipids

Phosphatidylcholine	Acyl Chain Length, No. of unsaturations	T_m	Reference
		°C	
DSPC	18:0, 18:0	55	Goodwin et al. (1982)
HSPC	16–18 (mixture)[a]	52	Horowitz et al. (1992)
DPPC	16:0, 16:0	42	Papahadjopoulos et al. (1973b)
POPC	16:0, 18:1	−7	Scherer and Seelig (1989)
SLPC	18:0, 18:2	−16.7	Sanchez-Migallon and Aranda (1996)
DOPC	18:1, 18:1	−21	Barton and Gunstone (1975)
eggPC	Mixture[b]	−2.5	Bach et al. (1982)
DSPG	18:0, 18:0	53.0	Surewicz and Epand (1986)
DPPG	16:0, 16:0	41.1	Boggs et al. (1989)
eggPG	Mixture	37	Vincent et al. (1991)
DSPA	18:0, 18:0	58	Krill et al. (1992)

An excellent database containing easily searchable physical properties of numerous lipids can be found at http://www.lipidat.chemistry.ohio-state.edu.

DOPC, dioloeylphosphatidylcholine; eggPG, egg phosphatidylglycerol; POPC, 1-palmitoyl, 2-oleoyl phosphatidylcholine; SLPC, 1-stearoyl, 2-linoleoyl phosphatidylcholine.

[a] Approximately 18% of the acyl chains are 16 carbons, and 82% are 18 carbons. All unsaturations have been reduced with a hydrogenation reaction.

[b] Contains ~34% of 16:0, ~1% of 16:1, ~10.5% of 18:0, ~31% of 18:1, ~17.7% of 18:2, ~3% of 20:4, and ~1.7% of other.

membrane packing and permeability considerations, and that the inclusion of high-phase transition anionic lipids into solid liposomes can actually increase circulation lifetimes.

However, as was stated previously, all cases must be considered individually. In one study with another anionic phospholipid, PA, liposomes composed of DSPC/1,2-distearoyl-3-*sn*-phosphatidic acid (DSPA)/Chol (3:1:4) were cleared ~10 times faster than DSPC/Chol liposomes (1:1; Senior, 1987). DSPA has a T_m value comparable to those of DSPG and DSPC at 58°C, and so for DSPA at least, liposome charge appears to become more important than membrane packing in the determination of rates of uptake. Of course, DSPA was introduced at 25% of the total phospholipid content and has two negative charges per molecule. In the previous example, DSPG was incorporated at only 10% of the total phospholipid and has only one negative charge, consequently adding an additional layer of complexity involving surface charge density. Janoff and coworkers have indeed shown a steep dependence of blood clearance rates on the mol% of the negatively charged component *N*-glutaryl-dipalmitoylphosphatidylethanolamine (*N*-glutaryl-DPPE) in DSPC liposomes (Ahl et al., 1997). The normalized AUC in plasma was greatest at 10 mol% *N*-glutaryl-DPPE and rapidly declined both below and above this value. The authors suggested the small amounts of negatively charged lipids stabilize neutral liposomes against an aggregation-dependent uptake mechanism. All of these examples point to the reality that different liposomes, and even comparable liposomes with different phospholipid headgroups of similar charge, may have very different mechanisms responsible for their uptake (Daemen et al., 1997).

An even more intriguing question is raised based on these analyses. Have CLs really been optimized? Are small DSPC/Chol liposomes really the most efficient liposomal carriers in the absence of steric stabilization? At least three studies have suggested that the inclusion of small amounts (10 mol%) of certain negatively charged lipids such as DSPG or *N*-acylated phosphatidylethanolamines (Park et al., 1992; Gabizon et al., 1996; Ahl et al., 1997) actually increase circulation $T_{1/2}$ even further. Additional studies will be needed to elucidate the exact nature of this stabilizing effect and determine more carefully the dependence of this stabilization on the structure of the stabilizing lipid and such parameters as membrane packing. Whether these liposomes would offer any improvements over SSLs remains to be seen, but at least one study has suggested that some DOX-loaded anionic liposome formulations demonstrate an efficacy similar to that of SSL DOX (Gabizon et al., 1996).

D. Effect of Membrane Packing Constraints on Pharmacokinetic Parameters

The effect of bilayer fluidity and the relative nature of the lipid components can have a considerable impact on the clearance from the circulation of both the liposome and the associated drug. These effects can either be direct effects, such as inhibition of penetration and thus binding of serum proteins (Papahadjopoulos et al., 1973b), or indirect effects, such as stabilization of the drug formulation to reduce the rate of drug leakage (*VII. Stability in Plasma and Storage*). The presence of Chol probably has one of the most important roles in the maintenance of membrane bilayer stability and long circulation times in vivo (Gregoriadis and Davis, 1979; Senior and Gregoriadis, 1982; Senior, 1987). In the absence of Chol, CLs are destabilized by HDL particles (Chobanian et al., 1979; Damen et al., 1980) and upon release, their components can be readily eliminated from the circulation. For liposomes with and without Chol, clearance rates were shown to negatively correlate with increased stability in plasma (Senior and Gregoriadis, 1982). The presence of steric stabilization makes the need for Chol less apparent for empty liposomes, but for drug-loaded liposomes, Chol is necessary for maintenance of the drug in the liposomal interior. The phospholipid component also plays a prominent role in the maintenance of high plasma levels of liposomes. DSPC/Chol and SM/Chol liposomes have higher $T_{1/2}$ values in the circulation compared with more fluid liposomes containing eggPC or even 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC; Gregoriadis and Senior, 1980; Senior, 1987). This is presumably due to the decreased affinity of these liposomes for serum opsonins required for their uptake. To be most effective, the PC component must have a phase transition that is significantly above 37°C. An inspection of the gel-to-liquid-crystalline phase transitions (T_m) of a variety of different PC molecules (Table 2) shows the T_m value for eggPC is below 37°C, whereas DPPC has a T_m value of only a few degrees above (with a pretransition at 37°C). However, both DSPC and HSPC have a T_m value that is ~15–17°C higher than 37°C. Thus, at 37°C, HSPC- and DSPC-containing liposomes have a considerably more rigid membrane bilayer that resists penetration of serum opsonins than do eggPC- or DPPC-containing formulations. It is no surprise, then, that these liposomes tend to be the most stable in the circulation and display the longest circulation lifetimes.

Sphingomyelin (SM) has an added effect on circulation lifetimes. SM/Chol and SM/DSPC/Chol liposomes were both shown to have longer circulation lifetimes than DSPC/Chol (Hwang, 1987; Allen et al., 1991), indicating an additional stabilizing effect of SM. SM can form intermolecular hydrogen bonds with neighboring Chol molecules (Schmidt et al., 1977; Sankaram and Thompson, 1990), resulting in greater stability and a decreased ability of plasma proteins to insert into liposomal membranes.

The rate of elimination of a liposomal drug from the circulation is also dependent on the rate of drug leakage from the carrier. Because drugs considered for liposome encapsulation often have circulation times significantly shorter than the liposomal carrier, premature release can lead to an apparent increase in the rate of elimination of the liposomal drug from the circulation. In DOX-loaded SSLs (SSL DOX) with HSPC, DPPC, or eggPC as the phospholipid component of the formulation, a correlation was observed among the phase transition (T_m) of the phospholipid component, the stability of the formulation in in vitro plasma stability tests, and plasma levels of the drug in vivo (Fig. 6 and Table 2; Gabizon et al., 1993). Liposomes containing high-phase transition lipids formed more stable formulations, which were better able to retain their drug and showed apparent increases in drug circulation lifetimes. A similar result was seen by Mayer and coworkers using CLs and DOX (Bally et al., 1990b). A more detailed explanation of the different factors responsible for maintaining a stable formulation of different drugs in the plasma is given in *VII. Stability in Plasma and Storage*.

The conclusions that can be drawn from these data differ for CLs and SSLs. For CLs, a membrane composed

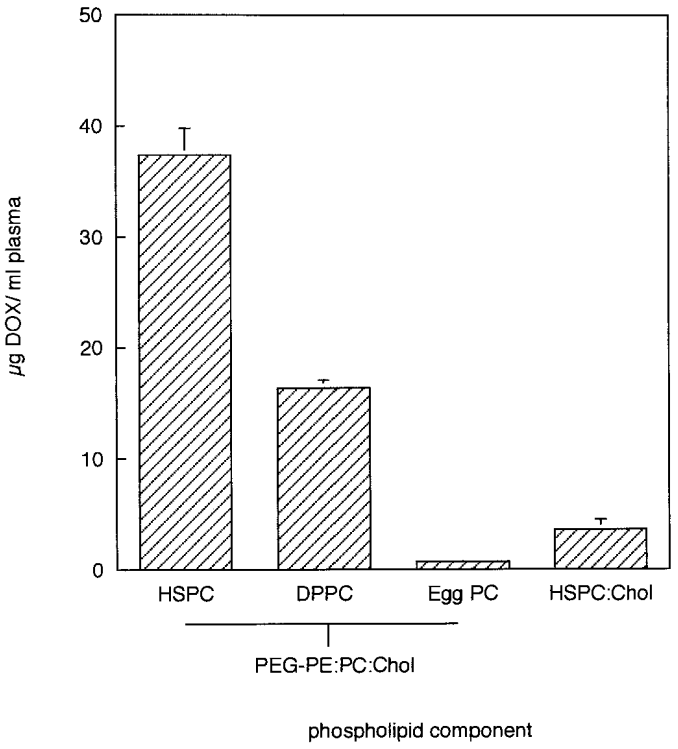


FIG. 6. Dependence of plasma drug levels on liposome composition. SSL DOX lipid formulations containing different species of phosphatidylcholine were injected into Sabra female mice (four per group) at a dose of 10 mg/kg DOX. The molar ratio of PEG-DSPE/PC/Chol was kept constant at 0.75:9.25:8 for each formulation, whereas the species of the PC component was varied. The different PCs used were HSPC, DPPC, and eggPC. HSPC/Chol (10:8) liposomes were used as a CL control. DOX measurements were taken 24 h after drug administration. This figure was adapted from Gabizon et al. (1993).

of Chol and high-phase transition phospholipids appears to be imperative for maintaining long circulation times and subsequent delivery of high levels of liposomes to solid tumors (see *III. Accumulation of Liposomal Drugs in Tumors*). SSLs are more pliable and can be used with fluid-phase lipids to obtain long circulation times and high tumor levels of liposomes. For both types of liposomes, the lipid composition of the liposome membrane is essential in maintaining a stable encapsulation of the drug while in the circulation. For most amphipathic drugs that are either weak acids or weak bases (the majority of classic chemotherapeutic agents), this is of considerable importance because these drugs will more rapidly leak from the carrier while in the circulation, unless high-phase transition lipids are used.

E. Effect of Steric Stabilization on Pharmacokinetic Parameters

Original attempts to mimic the surface of red blood cells by including the sterically hindered GM_1 or PI in liposome preparations led to the development of long-circulating liposomes (Allen and Chonn, 1987; Gabizon and Papahadjopoulos, 1988; Gabizon et al., 1990). Later, PEG-DSPE was substituted for GM_1 or PI (Klibanov et al., 1990; Allen et al., 1991; Papahadjopoulos et al., 1991). A common misconception is that the attachment of PEG to the surface of a liposome prevents liposome uptake by the RES; rather, it simply reduces the rate of uptake. One of the most significant advantages of SSLs is the nonsaturable, log-linear pharmacokinetics, as described perviously. SSLs likely resist uptake by the high-affinity, low-capacity RES macrophages, resulting in increased circulation lifetimes (Allen et al., 1995a). Like CLs, the primary site of accumulation for SSLs is also the spleen and liver (Huang et al., 1992). However, the rate of accumulation in these tissues is considerably slower than that for CLs. Plasma levels of PEG-DSPE containing liposomes are increased 2- to 2.5-fold over DSPC/Chol (2:1) CLs and 7- to 10-fold over eggPC/Chol (2:1) liposomes in mice (Fig. 5; Lasic et al., 1991; Woodle et al., 1992). As mentioned earlier, CLs containing anionic lipids or those containing unsaturated lipid components, such as eggPC, are removed more readily from the circulation than those containing high-phase transition phospholipids (SM/Chol or DSPC/Chol). However, in the presence of PEG-DSPE, liposomes containing some charged lipids or low-phase transition phospholipids are found in plasma after 24 h at similar levels to those containing neutral high-phase transition phospholipids (Fig. 5). The presence of steric stabilization thus allows for the rate of clearance to be relatively independent of the remaining lipid composition for "empty" liposomes (Lasic et al., 1991; Woodle et al., 1992).

This is not inclusive of all phospholipid components. Both GM_1 and PEG-DSPE were unable to prevent liposomes containing PS from being cleared rapidly from the circulation (Allen et al., 1988, 1991), indicating that some membrane components may confer a very powerful propensity for a type of liposome being recognized and taken up by the RES. In addition, although the lipids composing the majority of the liposome may not have a direct effect on the removal of the liposomal carrier itself, they may have an indirect effect on clearance of the encapsulated drug. As previously described, when amphipathic drugs such as DOX are loaded into these liposomes, the rate of leakage from the liposome can become the rate-limiting step for clearance of the drug from the circulation if liposomes are not optimized to prevent leakage. The dose independence of liposome clearance, reduced recognition and clearance of liposomes by the RES, and flexibility in lipid compositions that can provide considerable advantages for SSLs that make them more desirable for an assortment of different applications.

The mechanism by which steric stabilization of liposomes increases their longevity in the circulation has been extensively discussed (Lasic et al., 1991, 1992; Needham et al., 1992a, 1999; Allen, 1994; Lasic and Martin, 1995). The basic concept of this discussion has been that a flexible-chained hydrophilic polymer or a glycolipid, such as PEG or GM_1, which occupies the space immediately adjacent to the liposome surface ("periliposomal layer"), tends to exclude other macromolecules from this space. Consequently, access and binding of blood plasma opsonins to the liposome surface are hindered, and thereby interactions of RES macrophages with such liposomes are inhibited. The exclusion of extraneous macromolecules from the periliposomal layer creates steric hindrance ("steric stabilization effect"), which is manifested as increased interbilayer repulsive forces and results in an increased interbilayer separation of PEG-decorated bilayers compared with unmodified ones (Lasic et al., 1992b; Needham et al., 1992b). Ganglioside GM_1 has less effect on the interbilayer separation compared with PEG. Measurements of streptavidin-induced agglutination rate of biotinylated SSLs demonstrated that the steric barrier decreased with decreasing PEG chain length and at higher PEG lengths (PEG M_r = 1900 and 5000) was significantly greater than that produced by GM_1 (Mori et al., 1991).

Several studies have argued that the presence of PEG or GM_1 does in fact lead to both a decreased extent and rate of binding of plasma proteins to liposomes (Senior et al., 1991; Chonn et al., 1992; Blume and Cevc, 1993; Semple and Chonn, 1996), although direct experimental evidence is not abundantly clear for PEG-DSPE-containing liposomes. In two earlier studies in which decreased protein binding or opsonic activity was shown for SSLs, the incubation of PEG-stabilized liposomes with plasma components was completed in 2 to 15 min (Allen et al., 1994; Semple and Chonn, 1996). Another study showed similar findings in vivo after a 2-min incubation but approximately equivalent levels of bound protein for a CL and a PEG-stabilized liposome formulation after 30

min (Harvie et al., 1996). Chonn et al. (1992) have previously shown a correlation of protein-binding levels in CLs and GM_1-containing liposomes to in vivo circulation lifetimes, which is consistent with the observations seen with SSLs. Thus, decreased binding of serum opsonins by GM_1-containing liposomes can result in decreased opsonin activation, as has been shown for complement factor C3 (Chonn et al., 1992), or reduced uptake by macrophages (Wassef et al., 1991; Alving and Wassef, 1992). Finally, reduced clearance may be partially due to steric hinderance for the binding of liposome-bound opsonins to their receptors on RES macrophages (Klibanov et al., 1991; Mori et al., 1991; Allen, 1994). Allen and coworkers have suggested that the elimination of SSLs may occur via the same mechanism as CLs, after a slow removal of PEG-DSPE from the membrane (Allen et al., 1991; Allen, 1994). Blume and Cevc (1993) suggested that SSLs may simply require longer to bind the opsonins necessary for uptake by the RES. The net effect of these phenomena is that by sterically hindering the approach to the liposome surface by large molecules or cells, liposomes can attain longer circulation lifetimes, allowing them greater time to accumulate in tumors.

F. Comparison of Pharmacokinetic Parameters for Different Liposomal Formulations

SSL DOX has a long half-life in the circulation compared with free DOX (Table 3). Pharmacokinetic data for free DOX are best described by a biexponential fit with a rapid distribution phase and a slow terminal elimination phase. The majority of free DOX is eliminated in the initial rapid phase. With SSL DOX, a major portion of the plasma AUC is attributed to the prolonged terminal phase (Papahadjopoulos and Gabizon, 1995; Gabizon and Martin, 1997). Half-lives in rats were found to be between 22 and 23.6 h (Mayhew et al., 1992; Working and Dayan, 1996), whereas those in humans approximated 45 h (Gabizon et al., 1994). For conventional formulations in humans, the terminal $T_{1/2}$ is significantly shorter: from 6.7 to 25 h for TLC D-99 and from 2.8 to 8.3 h for DaunoXome. Although the $T_{1/2}$ was found to be independent of dose for SSL DOX, it increased with increasing dose for both DaunoXome and TLC D-99. Recently, the dose-independence of SSL DOX at very high concentrations of drug, 60 mg/m^2, was called into question when it was reported that a decreased rate of clearance was observed at these higher doses (Martin, 1998). This may result from a drug-induced toxicity to macrophages responsible for their elimination, similar to the effect described by others with CLs (Bally et al., 1990b; Parr et al., 1997). The increased $T_{1/2}$ values of liposomal drugs relative to free drugs were also consistent with a decreased rate of clearance of the liposomal carriers. DOX is cleared 560 times slower in humans when encapsulated in SSLs, and daunorubicin is cleared 35 to 56 times slower with DaunoXome, a CL formulation, compared with the free drugs. The stable encapsulation of drug within the liposome, combined with the large size of the carrier, likely prevents filtration and removal of the drug by the kidneys.

It should be emphasized that the AUCs that are typically referred to in this discussion are based on drug that is for the most part nonbioavailable. It is entrapped inside the carrier and unable to elicit any response. Thus, the term "AUC," which is commonly used by those in the liposome field of study, for liposomal drugs may appear a little misleading to some because it does not truly represent the pool of bioavailable drug in the plasma or in the tumor. Several studies have shown for DOX that >95% of the drug remains liposome associated in the plasma (Gabizon et al., 1994; Martin, 1998). A technical limitation in the ability to accurately measure the rate at which drug is released from the carrier has also prevented us from expressing true AUC measurements for bioavailable drug in the tumor. The AUC measurements typically being referred to are of total

TABLE 3
Pharmacokinetic parameters of SSL DOX in various animals

Animal Model	Formulation	Dose	$T_{1/2}$	AUC	V_d	CL
		mg/kg	*h*	*mg * l/h*	*ml*	*ml/h*
Rats[a]	SSL DOX	1.0	T_1 = 1.8 T_2 = 23.6	683	13	0.4
	Free DOX	0.9	T_1 = 0.16 T_2 = 29.1	11.1	1,014	24.3
Male Sprague-Dawley rats[b]	HSPC/Chol/PEG-DSPE (37:20:3)	6.0	T_1 = 2.8 T_2 = 22	2,769	18	0.5
	Free epirubicin	6.0	0.23	15	3,700	111
Rabbits[a]	SSL DOX	1.0	T_1 = 0.5 T_2 = 21.3	368	176	6
	Free DOX	1.0	T_1 = 0.03 T_2 = 4.07	1	13,651	2,536
Dogs[a,c]	SSL DOX	1.5	T_1 = 0.20 T_2 = 25.9	656	596	15.5
	SSL DOX	0.5	27 ± 5	304 ± 118	1000 ± 100	25.2 ± 7.2

T_1, half-life associated with the first exponent of elimination; T_2, half-life associated with the second exponent of elimination; V_d, volume of distribution; CL, total plasma clearance.
[a] Working and Dayan (1996).
[b] Mayhew et al. (1992).
[c] Gabizon et al. (1993).

drug, including both liposome and free drug. Depending on the lipid composition used and the rate of clearance in the particular organ or compartment being studied, these relative amounts may vary significantly.

The increased $T_{1/2}$ values of liposomal drugs translate into an increased AUC for Doxil in plasma compared with the free drug (Tables 3 and 4). In rats, the AUC for Doxil is >60 times that of free DOX, and this increase is elevated to a 368-fold increase in rabbits (Working and Dayan, 1996). In humans, the AUC is increased by 250 to 600 times in the case of Doxil over free DOX, 20 to 30 times in the case of TLC D-99, and ~60-fold in the case of DaunoXome (Table 4; Conley et al., 1993; Cowens et al., 1993; Gabizon et al., 1994). The smaller plasma AUCs for DaunoXome and TLC D-99 relative to Doxil may reflect both the shorter circulation times of the carriers, as a result of the lack of steric stabilization, and their increased rate of drug leakage. Even doses greater than three times those used for DOX were unable to provide comparable plasma levels of the relevant anthracycline (609 mg/*h/liter for 25 mg/m^2 DOX and 375.3 mg*h/liter for 80 mg/m^2 daunorubicin). For TLC D-99, plasma AUCs reached their maximum value at 50.5 ± 44.9 mg*h/liter, 10-fold lower than that for Doxil. Mayer and coworkers recently demonstrated they could obtain plasma AUCs for DOX in mice from 27 to 57% of those obtained with an SSL formulation by encapsulating DOX in DSPC/Chol liposomes and injecting them at the relatively high doses of 20 mg/kg DOX and 100 mg/kg lipid (Parr et al., 1997; Bally et al., 1998; Mayer et al., 1998). Assuming that a stable formulation can be prepared with SSLs (this is not always the case as will be seen with liposomal VCR), the data suggest that plasma drug levels, and thus the total AUC, will always be greater with SSL formulations. Although there is little debate on this particular point, there is a significant amount of debate over whether higher plasma levels necessitate a more favorable clinical outcome. This question is a complex one, and a considerable part of the remainder of this review focuses on how and whether this question can be answered.

The volume of distribution for free DOX is high in all species examined, indicating a wide tissue distribution. The small molecular size and amphipathic nature of the free drug allow it to rapidly distribute to both healthy and diseased tissues. However, when administered in liposomal form, the volume of distribution was reduced >60-fold to values approximating the plasma volume, suggesting that both DaunoXome and Doxil are restricted to the central compartment (Gabizon et al., 1993; Tables 3 and 4). The relatively large size of liposomal carriers (45–150 nm) prevents them from passing through the 2-nm pores found in the endothelium of blood vessels in most healthy tissues or even the 6-nm pores found in postcapillary venules (Seymour, 1992). In addition to the size of the carrier, the stability of the

TABLE 4
Pharmacokinetic parameters of CL and SSL DOX in humans

Formulation	Dose	$T_{1/2}$	AUC	V_d	Cl	Reference
	mg/m^2	*h*	*mg * h/l*	*l*	*l/h*	
SSL DOX	25	3.2 (0.2–5.4) 45.2 (20.8–59.1)	609 (227–887)	4.1 (3.0–6.5)	0.08 (0.05–0.21)	Gabizon et al. (1994)
	50	1.4 (0.2–7.3) 45.9 (29.3–74.0)	902 (335–2497)	5.9 (2.3–10.1)	0.09 (0.03–0.24)	Gabizon et al. (1994)
Free DOX	25	0.07 (0.05–0.09) 8.7 (3.6–13.3)	1.0 (0.7–1.3)	254 (126–393)	45.3 (39.7–48.6)	Gabizon et al. (1994)
	50	0.06 (0.06–0.08) 10.4 (5.4–26.8)	3.5 (2.6–6.0)	365 (131–501)	25.3 (13.3–35.2)	Gabizon et al. (1994)
SSL DOX	20	5.6 56.6	577	4.7	0.07	Working and Dayan (1996)
TLC D-99	20	0.71 ± 0.37 8.2 ± 6.2	30.4 ± 32.5	21.4 ± 14.0	23.5 ± 15.6	Cowens et al. (1993)
	25	0.29 ± 0.09 6.68 ± 2.94	19.7 ± 17.7	18.8 ± 10.7	23.3 ± 15.7	
	30	0.37 ± 0.16 25.0 ± 22.5	50.5 ± 44.9	8.2 ± 3.0	9.0 ± 7.8	
	90	0.45 ± 0.60 13.5 ± 6.6	14.1 ± 16.6	14.6 ± 7.8	21.8 ± 15.5	
TLC D-99	30	0.2 ± 0.1 25.1 ± 35.5	30.4 ± 25.8	7.1 ± 4.0	9.8 ± 10.7	Conley et al. (1993)
DSPC/Chol (2:1) (daunorubicin)	10	2.8	16.9	3.75	0.942	Gill et al. (1995)
	20	3.8	57.2	4.1	0.858	
	40	4.0	120.1	3.7	0.630	
	60	8.3	301.1	2.9	0.402	
	80	5.2	375.3	2.9	0.396	
Free daunorubicin	80	0.77	10.33	1055	13.38	Forssen and Ross (1994)
DSPC:Chol (55:45) (VCR)	2.0	1.95 ± 1.67 22.5 ± 9.85	6.5 ± 0.6	4.35 ± 0.84		Embree et al. (1998)
	2.8	7.2 ± 4.8 99.3 ± 17.2	40.2 ± 17.2	2.26 ± 0.47		

Errors are expressed as either a range (in parentheses) or as a S.D., depending on the source of the data. For expression of half-lives of elimination, the top value is the half-life associated with the first exponent of elimination, and the bottom value is the half-life associated with the second exponent of elimination.
$T_{1/2}$, half-life elimination of drug from the circulation; V_d, volume of distribution; Cl, total plasma clearance.

formulation can also have an effect on the volume of distribution. If drug leaks from the liposome before leaving the circulation, then the free drug can readily redistribute to healthy tissues. A comparison of Doxil or DaunoXome, both of which contain high-phase transition phospholipids (DSPC or HSPC), with TLC D-99, which contains the highly unsaturated eggPC, shows a significantly higher volume of distribution (4- to 5-fold at 25 mg/m^2) for the latter (Table 4). This indicates that DOX was released more rapidly from the carrier with TLC D-99 and has distributed more extensively into normal tissues than for more stable preparations with lower leakage rates. The consequences of this are an alteration in the toxicity profile and lower tumor levels of the drug. A more thorough review of the factors that contribute to the stability of a formulation in the plasma is given in *VII. Stability in Plasma and Storage.*

G. Tissue Distribution of Conventional and Sterically Stabilized Liposomes

Due in part to the size of the carrier, L-DOX has an altered tissue distribution compared with free DOX (Tables 5 and 6). Free DOX has a wide distribution, accumulating in most tissues to a significant extent. L-DOX preferentially accumulates in areas containing a discontinuous microvasculature, such as tumors, or in organs containing the macrophages of the RES, such as liver and spleen. This altered distribution reduces the concentration of drug at potential sites of toxicity, such as the heart. A comparison of the biodistribution of free drug and that encapsulated in both CLs and SSLs is given in Tables 5 and 6. When DOX levels are reported as peak levels of drug in various tissues, a significant increase in DOX is found in healthy tissues, such as kidneys, heart, and lung when administered as free DOX compared with both CL and SSL DOX (Table 5). L-DOX shows increased levels in blood, liver, spleen, and tumor (Table 5). In the liver, SSLs were found almost exclusively in Kupffer cells and rarely in the more abundant hepatocytes (Huang et al., 1992; Litzinger et al., 1994). This is consistent with the role of Kupffer cells in removing liposomes from the circulation and suggests less damage to liver tissue than if delivered to parenchymal cells. However, in addition to macrophages, other investigators have demonstrated a significant uptake of liposomes by a low-affinity, high-capacity system involving hepatocytes in a manner dependent on both the size of the liposomes and the presence of PEG-DSPE (Scherphof et al., 1994). The nature of this discrepancy is unclear but may involve problems in detection of liposomes in hepatocytes by some methods. From these data, it appears as though liposomes preferentially accumulate in tumor and tissues of the RES, whereas free DOX distributes more uniformly between the various tissues.

Although peak drug levels indicate L-DOX reaches healthy tissues to a reduced extent, when tissue drug

TABLE 5
Tissue distribution of DOX in animals treated with L-DOX

Animal Model	Liposome Formulation	Time	Dose[a]	Plasma	Spleen	Liver	Skin	Heart	Lung	Kidney
		h	*mg/kg*	*μg/ml*			*μg/g*			
Nude mice (N87 tumor implants)[b]	HSPC/Chol/PEG-PE (92.5:70:7.5)	72	10	12.89		15.84 ± 1.58	5.36 ± 0.35	2.37 ± 0.46		
Rats[c]	HSPC/Chol/PEG-PE (56.4:38.3:5.3)	24	1		7.18 ± 1.41	1.27 ± 0.11	0.46 ± 1.1	1.67 ± 0.55	1.59 ± 0.33	1.70 ± 0.11
	Free DOX	0.5	1		2.56 ± 0.33	1.67 ± 0.16	0.46 ± 0.06	2.21 ± 0.17	2.71 ± 0.49	3.61 ± 0.40
Female CD1 mice[d]	eggPC/Chol (55:45) 200 nm	5	20	14.3 ± 3.8	995.1 ± 284.7	134 ± 20.2		4.1 ± 2.2	16.5 ± 4.1	17.3 ± 6.2
	DSPC/Chol (55:45) 230 nm	5	20	27.4 ± 3.2	832.2 ± 64.8	137.5 ± 10.7		2.4 ± 1.2	11.4 ± 3.4	13.1 ± 2.1
	Free DOX	5	20	0.09 ± 0.04	39.0 ± 8.3	25.8 ± 3.7		15.5 ± 3.4	29.9 ± 5.8	35.5 ± 7.9
Female BDF1 mice[e]	DSPC/Chol (55:45) (100 nm)	C_{max}	10	140.5 ± 5.3	259.8 ± 7.8	68.7 ± 3.7		7.8 ± 1.5		30.9 ± 0.6
	Free DOX	C_{max}	7.5	2.7 ± 0.3	13.5 ± 1.4	59.5 ± 2.4		35.1 ± 1.8		98.5 ± 6.4

C_{max} is the peak concentration of drug achieved, and it varied as a function of time.
[a] Dose is given in mg/kg DOX.
[b] Gabizon et al. (1997).
[c] Working and Dayan (1996).
[d] Mayer et al. (1989).
[e] Krishna and Mayer (1997).
Errors are provided where available.

TABLE 6
Tissue AUC values after i.v. administration of various liposomal and free drugs

Animal Model	Liposome Formulation	Dose[a]	Plasma	Tumor	Spleen	Liver	Heart	Lung	Kidney
		mg/kg	*μg · h/ml*			*μg · h/g*			
BALB/c mice with C26 colon carcinoma[b,d]	DSPC/Chol/GM_1 (2:1:0.2)	[c]		662.2	1974	1299	83.25	141.5	241.8
	DSPC/Chol (2:1)	[c]		256.2	3348	1757	31.98	28.3	40.48
Female BDF1 mice[e]	DSPC/Chol (55:45)	10	1095.2	470.6	8923.8	2444.5	67		1265.6
	Free DOX	7.5	1.3	73.8	496.9	874.9	820.4		1258.1
BALB/c mice with colon C26 carcinoma[f]	PEG-PE/DSPC/Chol (6:47:47)	5	809.5	169.6	320.3	309.4	41.9	91.4	132.0
	DSPC/Chol (1:1)	5	342.8	50.1	365.5	341.4	41.2	67.5	72.4
	Free DOX	5	1	18.1	178.1	168.8	63.2	106.4	146.1
BALB/c mice with P1798 lymphosarcoma[g]	DSPC/Chol (2:1) (62 nm)	20	2275.6	2470.5	3596.2	693.6	265.1	685.0	1237.2
	Free daunorubicin	20	10	245.1	2213.4	335.5	249.9	612.2	976.8
BDF_1 mice[h] (mitoxantrone)	DSPC/Chol	10	1970		12630	4832		178	751
	DSPC/Chol/PEG-PE	10	4863		7242	4302		218	697

AUC was calculated from either 0–96 h,[d] 1–24 h,[f] by the trapezoidal rule,[e, h] or 0–48 h.[g]
[a] Dose is given in mg/kg DOX.
[b] Data were collected using encapsulated ^{67}Ga as the tracer. The tissue distribution is expressed as percent of injected dose per gram of tissue versus time (h).
[c] 10–15 μmol of phospholipid/kg.
[d] Huang et al. (1992). Data were collected using encapsulated ^{67}Ga as the tracer. The tissue distribution is expressed as percent of injected dose per gram of tissue versus time (h) after injection.
[e] Krishna and Mayer (1997).
[f] Unezaki et al. (1995).
[g] Forssen et al. (1992).
[h] Chang et al. (1997).

levels are reported as the AUC, even in healthy tissues, tissue drug levels approach those for free DOX (Table 6). DOX delivered via CLs accumulates to a reduced extent in non-RES tissues compared with delivery by SSLs. This is most likely a result of the reduced circulation lifetimes of CLs. The AUC for tumors is still between 2.5- and 10-fold greater than that for free DOX. A more detailed comparison of the extents of accumulation in tumors is given in *IIIB. Rate and Extent of Accumulation in Tumors*. In one of the earliest comparisons of SSLs (GM_1) versus CLs (Huang et al., 1992), liposome levels in tumor (followed with encapsulated ^{67}Ga) were greater than twice those of DSPC/Chol liposomes (Table 6). The levels in spleen and liver for DSPC/Chol liposomes were 1.35 to 1.7 times those of the GM_1-containing formulation, reflecting their more rapid uptake by the RES. In the three healthy non-RES tissues measured (heart, lung, and kidneys), liposome levels were significantly greater for the SSL formulation, suggesting that longer circulation also leads to higher AUCs for liposomes in healthy tissues. The few comparative studies with L-DOX or mitoxantrone showed either insignificant differences or slightly elevated AUCs in healthy tissues for SSLs relative to the CL formulation (Table 6; Unezaki et al., 1995; Chang et al., 1997). In all cases, the total AUC was either comparable or decreased for the liposomal form compared with the free drug. Considering that the overall exposure for these tissues is approaching equivalent amounts for free and encapsulated drug, it might be reasonable to expect the level of toxicity in these tissues to be similar. However, some acute toxicities are dependent on peak levels of the drug, and liposomal drugs accumulate in these tissues at a much slower rate than the free drug. In addition, the liposomal drug is not completely bioavailable, and thus the effective concentration of the drug in these tissues is considerably reduced. In one study, a histological section of cardiac muscle showed accumulation of liposomes only within blood vessels between muscle fibers and not within the muscle itself, indicating that liposomes were unable to extravasate in the heart to areas where they may do considerable damage (Working et al., 1994). This is discussed in greater detail in *VI. Toxicology of Liposomal Chemotherapy*.

H. Metabolism and Elimination of Liposomal Doxorubicin

Anthracyclines are metabolized in human plasma to a variety of both active and inactive metabolites (Takanashi and Bachur, 1976; Fig. 7). The reduction in DOX by an aldo-ketoreductase results in the formation of the most prominent metabolite, doxorubicinol (**II**), in plasma, bile, and urine (Takanashi and Bachur, 1976). A two-electron reduction in DOX with subsequent elimination of the sugar results in the inactive metabolite, a 7-deoxyaglycone (**V**: Takanashi and Bachur, 1976; Doroshaw, 1996). In several studies, researchers looked for the presence of DOX metabolites in plasma and urine after the administration of L-DOX (Gabizon et al., 1991, 1994; Northfelt et al., 1996). Although several of the more common metabolites (doxorubicinol and glucoronide and sulfate derivatives of 4-dimethyl,7-deoxyaglycones) were observed in urine, they were at diminished levels (2.5%) compared with the administration of free DOX (11%; Gabizon et al., 1994). In two separate studies (Gabizon et al., 1994; Northfelt et al., 1996), doxorubicinol (**II**) was not observed in plasma at any time after the administration of SSL DOX. This is not surprising considering that the liposomal membrane protects its contents from inactivation by plasma enzymes. The importance of a stable formulation is essential in

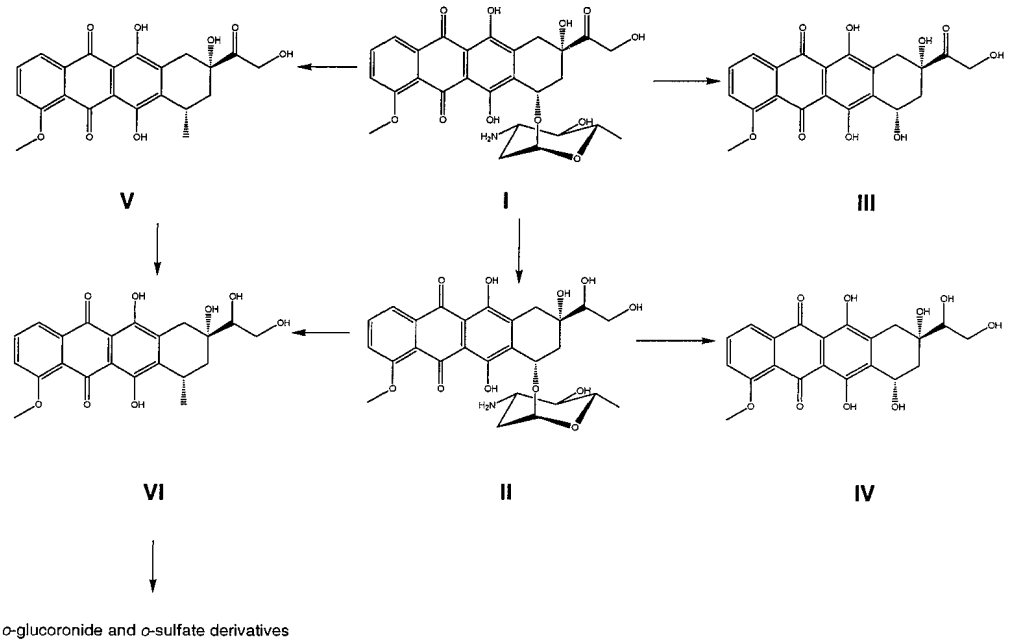


FIG. 7. Metabolism of DOX in vivo. DOX (**I**) can be converted to either inactive (deoxyaglycones; **III** or **V**) or active (doxorubicinol; **II**) metabolites in the circulation. Initially, DOX is converted to doxorubicinol (**II**), DOX aglycone (**III**), or deoxydoxorubicin aglycone (**V**), although the preferred pathway is for the metabolism to **II**. Doxorubicinol (**II**) is the primary metabolite found in both plasma and urine. Doxorubicinol can be further metabolized to doxorubicinol aglycone (**IV**) or deoxydoxorubicinol aglycone (**VI**), with metabolism to **VI** being the preferred pathway. Finally, **VI** can be converted to more polar metabolites such as *o*-sulfate or *o*-glucoronide derivatives. A notable advantage of liposomes is that they are able to protect their contents from metabolism and inactivation in the circulation, thus allowing higher levels of the parent compound to arrive at the tumor site. This figure was modified from Takanashi and Bachur (1976).

maintaining this advantage. In formulations containing unsaturated phospholipids, DOX leaks rapidly from the liposome and doxorubicinol was detected in plasma at times as short as 30 min (Gabizon et al., 1991; Embree et al., 1993). Although small amounts of DOX metabolites have been observed in tumor and tumor exudates (Gabizon et al., 1994; Siegal et al., 1995), its protection from inactivation by plasma enzymes almost certainly increases the percentage of drug that arrives in the active form at the tumor site.

L-DOX is eliminated in the urine at a much slower rate than free DOX (Vaage et al., 1998). In a mouse model, free DOX was found in urine samples as readily as 15 min and up to 48 h after the administration of the drug. SSL DOX was not detected in the urine until almost 1 h and could still be detected up to 5 days after drug administration. This is consistent with a controlled-release mechanism for the liposome-encapsulated drug, where the drug is released from its carrier at a very slow rate.

An understanding of the mechanisms responsible for maintaining high circulating levels of drug in the plasma is essential to design carriers that remain in the circulation sufficiently long to have a high probability of accumulating in tumors. Nevertheless, long circulation time is only one aspect of liposomes that results in their preferential antitumor activity. If liposomes were unable to preferentially accumulate in tumors, they would be useful only as a controlled-release type of therapy. This is increasingly available through mechanical means, and thus there is minor clinical importance for the development of liposomes for this purpose. However, the fact that liposomes do accumulate preferentially in tumors allows them to be passively targeted and gives rise to substantial increases in antitumor efficacy. In *III. Accumulation of Liposomal Drugs in Tumors*, we review the various mechanisms responsible for the uptake of liposomes into tumors and how they may be exploited for further increasing drug delivery to tumors in the future.

III. Accumulation of Liposomal Drugs in Tumors

A. Mechanistic Rationale for Liposome Accumulation in Tumors: Enhanced Permeability and Retention Effect Phenomenon

The accumulation of liposomes or large macromolecules in tumors is a result of a "leaky" microvasculature and an impaired lymphatics supporting the tumor area (Matsumura and Maeda, 1986, 1989; Huang et al., 1992; Seymour, 1992; Yuan et al., 1994; Jain, 1996). This effect is often referred to as the enhanced permeability and retention effect ("EPR phenomenon"; Matsumura and Maeda, 1986, 1989). With gold-labeled liposomes, both extravasation and transcytosis of liposomes in Kaposi's sarcoma-like dermal lesions were demonstrated (Huang et al., 1993). The principal pathway for the movement of liposomes into the tumor interstitium is via extravasation through the discontinuous endothelium of the tumor microvasculature, and transcytosis is thought to be a relatively minor pathway. Once in the

tumors, nontargeted liposomes are localized in the interstitium surrounding the tumor cells (Huang et al., 1992; Yuan et al., 1994). Liposomes were not seen within tumor cells, although they were observed in resident tumor macrophages. The limited distribution of liposomes within the tumor interstitium results from a high interstitial pressure and a large interstitial space compared with normal tissues (Jain, 1989, 1990). Large tumors are more difficult to treat than small ones, in part because of the resulting increase in interstitial pressure, which prevents access of drugs to the necrotic core (Jain, 1990). Recently, liposomes were shown to penetrate the tumor more uniformly after the addition of an internalizing anti-HER2 Fab' fragment to the liposome surface (Papahadjopoulos et al., 1999) or by combining liposomal delivery with local hyperthermia. The extravasation and accumulation of liposomes into tumors are depicted in Fig. 8. Targeting to endocytic pathways may also increase the bioavailability of some drugs by degrading the liposomal carrier in the late endosome or lysosome. These advances are discussed in more detail in *VIII. Bioavailability of Encapsulated Drug*.

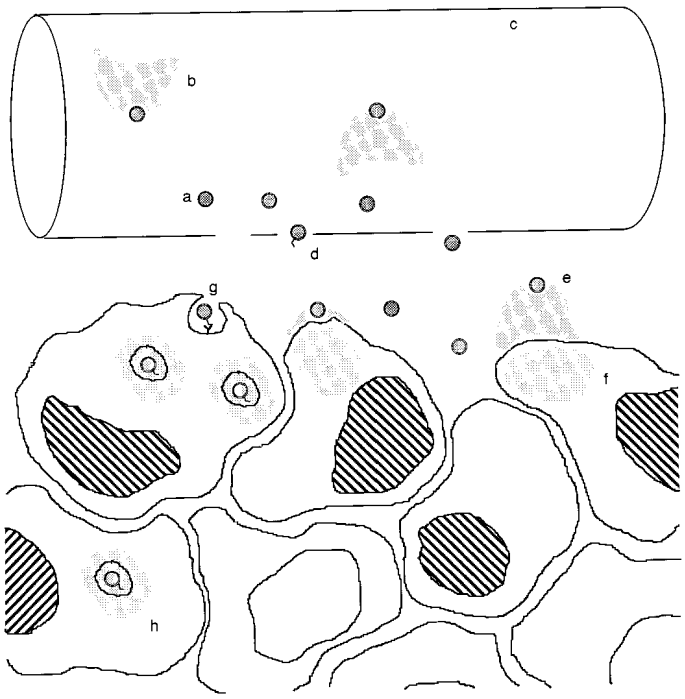


FIG. 8. Scheme showing accumulation of liposomes and drug in tumors. a, slow-release liposomes are able to carry some drugs securely in the circulation. b, rapid-release liposomes leak their drug, to a greater extent in the circulation. The drug is then free to diffuse and take on the pharmacokinetics of the free drug. c, the continuous endothelium of healthy tissues prevents leakage of liposomes into these tissues. d, however, the tumor vasculature is discontinuous with gaps ranging from 100 to 780 nm, allowing liposomes to extravasate and reach the tumor interstitium. e, nontargeted liposomes accumulate in the tumor interstitium, where they eventually leak their drug. f, DOX can then enter nearby tumor cells and accumulate in the nucleus where it elicits its cytotoxic effects. g, targeted liposomes also accumulate in tumors, but on entering the tumor area are endocytosed by tumor cells. This likely results in breakdown of the carrier in the lysosome and an increased delivery of DOX to the nucleus. h, in addition, HER2-targeted liposomes have been shown to distribute within the tumor, increasing the access of DOX to tumor cells deep within the tumor mass. Hyperthermia in combination with SSL DOX was shown to result in a similar distribution (not drawn to scale).

The rate of accumulation and subsequent removal of liposomal drugs are affected by a variety of factors. The absence of functioning lymphatics, in combination with a high interstitial pressure, results in the trapping of liposomes within the tumor area (Yuan et al., 1994). The result is a relatively slow rate of elimination from the tumor. Several reports suggest that the observed elimination of L-DOX from the tumor is more likely due to the release of free drug from the carrier and its subsequent metabolism and diffusion from the tumor. In a brain tumor model, 7-deoxyaglycone metabolites were observed at 96 and 120 h after injection, when tumor drug levels were starting to decrease (Siegal et al., 1995). In a separate experiment, tumor levels of DOX and a nonexchangeable lipid marker, [^{3}H]cholesteryl hexadecyl ether, were measured at identical times (Goren et al., 1996). Although the lipid marker continued to accumulate over the entire time course, 100 h, to a maximum of ~6% of the injected dose/g of tumor, DOX levels reached a maximum after 24 h of ~5% of the injected dose/g tumor and then slowly decreased. This suggests that after the trapping of the liposomal carrier in the tumor area, DOX is made bioavailable and attains its own separate rate of elimination.

1. Effect of Microvasculature Physiology. Liposomes are able to enter tumors due to a discontinuous tumor microvasculature, where pore sizes vary between 100 to 780 nm in size (Yuan et al., 1995; Hobbs et al., 1998). The junctions in the vascular endothelium of healthy tissues vary depending on the type of tissue (Seymour, 1992). In most tissues, including connective tissue and tissues of the muscle, heart, brain, and lung, intercellular tight junctions result in openings of <2 nm. These openings can approach 6 nm in postcapillary venules and are considerably smaller than the size of liposomal carriers (65–125 nm; Seymour, 1992; Lum and Malik, 1994). Organs or tissues with discontinuous endothelium, such as the fenestrated endothelium of the kidney glomerulus or the sinusoidal endothelium of the liver and spleen, can have junctions ranging from 40 to 60 nm for the former and up to 150 nm for the latter (Seymour, 1992). Most liposome formulations are larger than the threshold required for glomerular filtration. As described in *II. Pharmacokinetics and Biodistribution of Liposomes and Liposomal Drug*, it is the macrophages residing in the liver and spleen that are responsible for the removal of liposomes from the circulation and, thus, the other two major sites of accumulation. However, unlike in tumor tissue, where they become effectively trapped, if liposomes are able to avoid uptake by macrophages, then they are free to pass in and out of the liver and spleen. The selective accumulation in tumors is thus made possible by the impervious nature of the endothelium of most healthy tissues.

Vascular permeability in tumors is heterogeneous with respect to tumor type, location of the vessel within the tumor, and the tumor microenvironment (Yuan et al., 1994; Fukumura et al., 1997; Hobbs et al., 1998). The enhanced permeability and retention effect has been described for a variety of large macromolecules and drug carriers (Matsumura and Maeda, 1986, 1989; Maeda, 1991; Huang et al., 1992; Seymour, 1992; Yuan et al., 1994). Permeability and angiogenesis are also dependent on various growth factors and the microenvironment from which those growth factors are released or act on (Collins et al., 1993; Roberts and Palade, 1995; Dellian et al., 1996). The best studied of these is vascular permeability factor, also known as vascular endothelial growth factor. Vascular permeability factor also is an angiogenic factor; it helps to recruit new blood vessels to support the tumor (Folkman, 1985; Collins et al., 1993; Roberts and Palade, 1995). Basic fibroblast growth factor was shown in another study to increase vascular permeability, although its effects may be secondary to those of vascular endothelial growth factor (Dellian et al., 1996). The availability of these growth factors in different tumor environments will affect the accumulation of large macromolecules and liposomal carriers in tumors.

2. Blood-Brain Barrier. The blood-brain barrier represents a formidable barrier for drug delivery to the central nervous system. Tight junctions, the lack of fenestrations, and a low transcellular pinocytic index severely limit the accumulation of macromolecules in the brain (Levin et al., 1980; Seymour, 1992). Surprisingly, several groups have been able to show that even tumors located in the brain have a "leaky" microvasculature, although pore sizes are significantly smaller (100–380 nm) than those seen with tumors located elsewhere in the body (200–780 nm; Siegal et al., 1995; Hobbs et al., 1998). In a brain tumor model, Gabizon and coworkers were able to show high levels of SSL DOX accumulation in the tumor (Siegal et al., 1995). Fischer rats injected with SSL DOX at a dose of 6 mg/kg showed maximal accumulation at 48 h of 10 to 11 μg DOX/g of tumor tissue. This was 15-fold higher than the levels observed after the administration of an identical dose of free DOX (0.8 μg/g at 4 h), which were not different from levels found in the normal brain (contralateral hemisphere). There was no accumulation of SSL DOX in the contralateral hemisphere, and in brain tissue immediately adjacent to the tumor, levels were <2 μg/g tissue up to 70 h after injection but gradually increased to a maximum of 4 μg/g at 120 h. These results suggest that even in the tightly regulated central nervous system, a high tumor vascular permeability can be exploited for carrier-mediated drug delivery.

B. Rate and Extent of Accumulation in Tumors

The rate and extent of drug accumulation in tumors vary depending on dose, formulation, and tumor type (Table 7; Gabizon et al., 1996; Goren et al., 1996; Harasym et al., 1997; Parr et al., 1997). Drug accumulation in tumor is most often measured at single time points, commonly either 24 or 48 h. In some studies, the free drug is measured at shorter times, such as 1 h, due to its earlier peak of accumulation. Data comparing the degree of drug accumulation in tumors for free and liposomal drug at single time points are given in Table 7. Peak DOX levels are 3- to 15-fold greater in tumors when delivered via liposomes compared with the free drug. A comparison of CL versus SSL DOX showed an approximately equivalent accumulation in three tumor models when administered at high doses (20–55 mg/kg; Mayer et al., 1997; Parr et al., 1997; Table 7). When administered at lower doses (5–10 mg/kg), SSLs accumulated to a greater extent in tumors (Huang et al., 1992; Unezaki et al., 1995; Gabizon et al., 1996). The similar accumulation in the first study is probably an underestimate due to the taking of 24 h as the only or final time point, whereas significant tumor accumulation with SSL DOX occurs after this time. The effect of both dose and formulation is indirectly a result of their effect on liposome circulation lifetimes and on formulation stability. As described in *II. Pharmacokinetics and Biodistribution of Liposomes and Liposomal Drug*, the kinetics of SSL clearance are log linear and dose independent, which allow for significant concentrations of the liposomal drug to be in the circulation, even at low doses (Allen et al., 1995a). CLs, on the other hand, display saturable dose-dependent kinetics that result in rapid clearance of the liposomal drug at lower doses but a much slower rate of clearance, and thus higher blood levels at higher doses (Hwang, 1987; Allen et al., 1995a). It is postulated that at these lower doses, the large differences in circulation lifetimes between SSLs and CLs would result in a larger reservoir of liposomes available to enter the tumor in the case of SSLs. However, at higher doses, as saturation of the mechanisms responsible for liposome clearance occurs, the extent of these differences should be reduced. Using a variety of different lipid compositions with varying circulation lifetimes, a good correlation was observed between increasing lifetimes and high liposome levels in tumors (Gabizon and Papahadjopoulos, 1988). This relationship may prove to be overly simplistic. As factors such as drug-induced RES blockade and high liposome dose bring clearance rates of differing formulations closer together, poorly understood effects of liposome composition and physical properties (e.g., size) on rates of extravasation may begin to become important. Recent evidence suggests that CL formulations may accumulate in tumors at a more rapid rate than SSLs, but due to lower circulation lifetimes, they give rise to lower overall extents of accumulation (Gabizon et al., 1996; Mayer et al., 1998).

Gabizon et al. (1996) were the first to show that liposomes with decreased circulation lifetimes may accumulate in tumors at a more rapid rate. Later, Mayer and

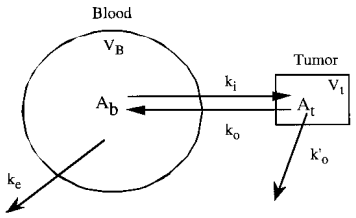
TABLE 7
Accumulation of liposomes in tumors

Animal and Tumor Model	Formulation	Dose	Time	Accumulation
		mg/kg	*h*	*μg/g*
$CD2F_1$ mice (P-1798 lymphosarcoma)[a]	DaunoXome	20	24	39.57 ± 15.38
	Free daunorubicin	20	24	2.02 ± 1.42
B6C3F1 mice (MA16C mammary adenocarcinoma)[a]	DaunoXome	20	24	19.98 ± 8.77
	Free daunorubicin	20	24	8.41 ± 1.11
Fischer rats (fibrous histiocytoma)[b]	Doxil	6	48	10.92
	Free DOX	6	4	0.8
Female C3H/HeJ mice (FSa-R fibrosarcoma)[c]	DSPC/Chol (55:45), 100 nm	20	24	16.3 ± 2.3
	DSPC/Chol/PEG-DSPE (50:45:5)	20	24	15.7 ± 1.7
	Free DOX	20	1	7.8 ± 3.1
Female C3H/HeJ mice (FSa-N fibrosarcoma)[c]	DSPC/Chol (55:45), 100 nm	20	24	23.9 ± 2.7
	DSPC/Chol/PEG-DSPE (50:45:5)	20	24	32.1 ± 4.7
	Free DOX	20	1	12.8 ± 1.2
BDF1 mice (Lewis lung carcinoma)[d]	DSPC/Chol (55:45), 100 nm	55	168	143.8 ± 18.2
	DSPC/Chol/PEG-DSPE (50:45:5)	55	168	133.0 ± 11.9
Shionogi mice (SC115 mammary carcinoma)[e]	TLC D-99	13	24	10.2 ± 3.6
		6.5	24	5.5 ± 1.1
		6.5	24	1.9 ± 0.08
BALB/c mice (M109 carcinoma)[f]	Doxil	5	48	6.5 ± 4
		10	48	16 ± 17.3
		20	48	42 ± 39.3
	Free DOX	5	3	3.6 ± 2.1
		10	3	5.2 ± 2.1
BALB/c female mice (J6456 ascites)[g]	PEG-DSPE/HSPC/Chol	10	24	5.9 ± 3.4
	DSPG/HSPC/Chol	10	24	4.2 ± 2.95
	HSPI/HSPC/Chol	10	24	5.3 ± 1.4
	HSPC/Chol	10	24	1.8 ± 0.13
	Free DOX	10	24	<0.05
BALB/c female mice (M-109 carcinoma)[g]	PEG-DSPE/HSPC/Chol	10	24	17.4 ± 8.8
	DSPG/HSPC/Chol	10	24	17.4 ± 6.1
	HSPC/Chol	10	24	19.7 ± 5.0
	Free DOX	10	24	2.2 ± 0.6
		mg/m²		
Humans (various carcinomas)[h]	Doxil	25	3–7 days	0.44
		50	3–7 days	0.69
	Free DOX	25	4–24	0.051
Humans (Kaposi's sarcoma lesions, biopsy specimens)[i]	DaunoXome	20	24	1.07
		40	24	1.06
Humans (Kaposi's sarcoma lesions, biopsy specimens)[j]	Doxil	10	72	2.06 ± 0.42
		20	72	1.61 ± 0.80
		40	72	7.71 ± 2.72
	Free DOX	10	72	0.18 ± 0.07
		20	72	0.31 ± 0.16
		40	72	0.82 ± 0.18

The composition, size, and drug/lipid ratio for daunorubicin and DOX are listed in Table 1. When available, the time of analysis was the time of maximal accumulation.
[a] Forssen et al. (1992).
[b] Siegal et al. (1995).
[c] Mayer et al. (1997).
[d] Parr et al. (1997).
[e] Mayer et al. (1990a).
[f] Gabizon et al. (1997).
[g] Gabizon et al. (1996).
[h] Gabizon et al. (1994).
[i] Forssen and Ross (1994).
[j] Northfelt et al. (1996).

coworkers proposed the use of a factor termed "tumor accumulation efficiency" (T_e), defined as the AUC of the drug in the tumor divided by the AUC in the plasma, to determine the efficiency of extravasation for a given liposome formulation (Mayer et al., 1997, 1998; Parr et al., 1997). In several different tumor models, the T_e value was 1.5- to 3-fold higher for DSPC/Chol formulations than the sterically stabilized equivalent (Mayer et al., 1997, 1998; Parr et al., 1997). The effect of steric stabilization on the rate of tumor accumulation is still controversial; at least one study that used videomicroscopy to follow fluorescently labeled liposomes showed a higher permeability for SSLs (Yuan et al., 1994). In another study, CL DSPC/Chol (55:45) liposomes delivered at 20 mg/kg to two fibrosarcoma models showed elevated drug levels in the tumor for CLs at 4 h but for SSLs at 24 h (Mayer et al., 1997). The initially increased rate of accumulation may be due to a higher permeability of the tumor microvasculature to CLs, but the later increase in SSL accumulation likely reflects the disappearing pool of CLs in the circulation, relative to SSLs.

The role of long-circulating properties and tissue uptake rate on the expected efficacy of a liposomal drug after i.v. bolus administration may be explored using a two-compartment open pharmacokinetic model (Scheme 1; Welling, 1986).



SCHEME 1.

The amount of liposomes in the blood compartment (A_b) and in the selected tissue [e.g., tumor (A_t)] is governed by the following set of equations and boundary conditions:

$$dA_b/dt = -(k_e + k_i)A_b + k_oA_t \quad (1)$$

$$dA_t/dt = k_iA_b - k_oA_t \quad (2)$$

$$A_b(0) = A_0; \quad A_b(\infty) = 0 \quad (3)$$

$$A_t(0) = A_t(\infty) = 0 \quad (4)$$

This set of linear differential equations with constant coefficients allows simple analytical solution resulting in somewhat cumbersome formulas for $A_b(t)$ and $A_t(t)$ (Welling, 1986). Assuming that drug efficacy correlates with the tissue exposure to the liposomes, which, in turn, is characterized by AUC, we need only calculate AUC for blood and tumor:

$$AUC_b = 1/V_b\int_0^\infty A_b dt \quad (5)$$

$$AUC_t = 1/V_t\int_0^\infty A_t dt \; AUC_t = \quad (6)$$

where V_b and V_t are physical volumes of the blood and tumor compartments, respectively.

After integration of right and left parts of eqs. 1 and 2 from zero to infinity and applying boundary conditions, we obtain:

$$(k_e + k_i) \cdot V_b \cdot AUC_b - k_o \cdot V_t \cdot AUC_t = A_0 \quad (7)$$

$$k_i \cdot V_b \cdot AUC_b - k_o \cdot V_t \cdot AUC_t = 0 \quad (8)$$

and, finally:

$$AUC_t = A_0 \cdot k_i/(k_e \cdot k_o \cdot V_t) \quad (9)$$

$$T_e = (AUC_t)/(AUC_b) = (k_i \cdot V_b)/(k_o \cdot V_t) \quad (10)$$

In these equations, "blood elimination" first order rate constant k_e includes all processes that lead to the removal of the carrier from the blood, including excretion, phagocytic clearance, and distribution into organs and tissues other than the tumor. Interestingly, T_e, perhaps deceptively termed "tumor accumulation efficiency" (Mayer et al., 1997, 1998), does not include liposome longevity in the circulation (characterized by k_e) as a factor and is determined essentially by the liposome uptake rate into the tumor. On the contrary, tumor AUC correlates not only with the liposome uptake (uptake rate constant k_i) but also with the liposome longevity in circulation (blood elimination constant k_e). These equations point out the importance of another process, often neglected: the rate of liposome elimination from the tumor (k_o). Liposome elimination from the tumor, which appears to be slower than tumor uptake and blood elimination, is poorly understood and awaits adequate experimental investigation.

The above simple model of liposome pharmacokinetic behavior can be modified for the case in which liposomes are not recycled from the tumor into the blood but instead are metabolized in situ. In this case, the effects of liposome uptake rate by the tumor and circulation longevity on the tumor AUC are similar to the previous model:

$$AUC_t = A_0k_i/(k_e + k_i)/(k'_oV_t) \quad (11)$$

and the expression for T_e is exactly the same as eq. 10.

Thus, within the framework of these models, circulation longevity of liposomes is not a factor in tumor accumulation efficiency, T_e, but rather is an important factor in the determination of overall tumor exposure to the drug.

In light of this controversy, a complicated question remains. If the permeability is increased for CLs, what is the overall significance of a 1.5- to 3-fold increase in permeability, taking into consideration the difference in circulation lifetimes? As expected, when the T_e was calculated for free DOX at 24 h after injection, the result was a 2.8-fold increase over that for an HSPC/Chol formulation (Gabizon et al., 1996). This is not surprising considering the small molecular size of the free drug and its rapid redistribution into tissues. However, the extent of accumulation in the tumor is greater for the liposomal formulation, as is the tumor AUC calculated at extended times. The overall tumor AUC appears to be a more relevant indicator of the effectiveness of a drug delivery system in liposomes where drug release from the liposome is similar. Mayer et al. (1998) showed two examples where the tumor AUC was slightly greater for CL formulations. One of these examples compared SM/Chol versus SM/Chol/PEG-PE formulations of VCR, where the concentrations of PEG-DSPE used were shown to result in a destabilization of the formulation and, consequently, lower amounts of drug available for delivery to the tumor (Webb et al., 1995). A second example was completed with DOX-loaded liposomes (Mayer et al., 1997). An important consideration when considering at total accumulation in tumors is the duration in which the AUC was calculated. The tumor AUC levels for all the examples given were calculated over the time period of 0 to 24 h. Other work, with substantially lower doses of SSL DOX, has shown that maximum tumor accumu-

lation does not occur until 48 h or longer in some cases and that a substantial portion of the tumor drug level-versus-time curve exists after 24 h in all cases (Papahadjopoulos et al., 1991; Vaage et al., 1994a; Siegal et al., 1995; Gabizon et al., 1996, 1997; Goren et al., 1996). In addition, the peak time of accumulation is conceivably even later considering the large doses (20 mg/kg) used in these studies. Thus, the tumor AUC levels reported by Mayer and coworkers are likely biased in favor of CLs by limiting the calculation to the first 24 h. Finally, although it is theoretically possible to increase circulation lifetimes to a sufficiently high level to achieve an advantage from the proposed increased rate of extravasation, the conditions required to do so may not be pharmacologically relevant. The doses required to obtain the needed circulation lifetimes (20–55 mg/kg) are 4 to 20 times larger than those being used in current studies with SSL DOX and will likely result in substantial toxicity if administered in multiple doses (see *VI. Toxicology of Liposomal Chemotherapy*).

Although differences in the rates of extravasation may not be sufficiently great to obtain a true advantage for CLs over SSLs, it is nevertheless an important parameter to keep in mind when designing drug delivery systems. Liposome size may be another important parameter that affects accumulation in tumors. Several studies have shown that the permeability of large macromolecules is independent of size as long as the translocating molecule is much smaller than the pore size in the endothelium of the tumor microvasculature (Yuan et al., 1995; Hobbs et al., 1998). However, even small liposomes (100 nm) are between 13 and 100% of the average pore size found in tumor endothelium, and liposomes (100 nm) have already been shown to have a reduced permeability compared with fluorescently labeled BSA molecules. Although a study with carefully sized liposomes has yet to be completed, theoretically these data suggest that even small changes in liposome size (50 nm) may significantly affect the rate of accumulation in tumors. A DSPC/Chol (2:1) formulation containing daunorubicin shows maximal accumulation in a lymphosarcoma solid tumor mouse model at 8 h with a subsequent elimination rate similar to free daunorubicin (Forssen et al., 1992). This initial rate of drug accumulation in this study is far more rapid than that seen with other CL or SSL DOX formulations (Gabizon et al., 1997; Mayer et al., 1997) and may be due to the smaller size (50 nm compared with 100 nm for the DOX-loaded liposomes), although differences in tumor type or drug-leakage rates cannot be ruled out. In situ fluorescence measurements for an identical small formulation showed a slower rate of accumulation, similar to that of other larger CL formulations (Forssen et al., 1996), thus complicating this interpretation. A carefully designed study that considered the effect of liposome size (50-nm increments) on tumor accumulation rates or vascular permeability would help test this hypothesis.

C. Hyperthermia and Vascular Permeability Factors for Increasing Vascular Permeability

Differences in vascular permeability have been exploited in two different ways to increase accumulation of liposomes in diseased tissues. Permeability of the tumor microvasculature was increased with the use of local hyperthermia, resulting in increased tumor levels of SSL DOX (Huang et al., 1994; van Bree et al., 1996). Hyperthermia can also result in increased rates of drug release from specially engineered thermosensitive liposomes (see *VIIIC. Hyperthermia and Thermosensitive Liposomes*; Gaber et al., 1995, 1996). In a second example, substance P was used in an inflammation model to increase vascular permeability and, thus, the extents of liposome accumulation (Rosenecker et al., 1996; Zhang et al., 1998). In both of these examples, increased accumulation in the diseased tissue resulted from a direct effect on the tumor microvasculature. It may prove advantageous in future studies to increase accumulation in tumors by altering liposome surface properties or vascular permeability directly to promote extravasation.

D. Sterically Stabilized versus Rapid-Release Conventional Liposome Carriers

In addition to using liposomes as slow-release liposomal carriers, such as SSL DOX, they can be used as rapid-release systems, such as TLC D-99. The low-phase transition phospholipid component, eggPC, of rapid-release liposomal carriers allows the drug to leak more quickly from the liposome, at least partially while in the circulation (Bally et al., 1990b; Gabizon et al., 1993). Although slow-release liposomal carriers accumulate in tumors on a time scale similar to or greater than the release of DOX from the carrier, rapid-release systems can release their drug to a greater extent before reaching the tumor, where it can diffuse into the tumor as the free drug. It should be emphasized that "slow" and "rapid" are relative terms, and the magnitude of the leakage rates will ultimately be determined by the physicochemical properties of both the drug and the carrier. The release of DOX in the plasma for TLC D-99 can be demonstrated by considering the drug/lipid ratio, which drops from 0.29 to <0.05 μg DOX/μg lipid in 24 h (Harasym et al., 1997). Approximately 58% of the drug was released from the carrier within the first hour. The differences in tumor accumulation show that the drug slowly accumulates in tumors when delivered via slow-release liposomal carriers (Papahadjopoulos et al., 1991; Bally et al., 1994; Gabizon et al., 1996, 1997), likely reflecting delivery of the intact liposome-encapsulated drug. In rapid-release CL liposomal carriers, DOX accumulated rapidly in the tumor and levels remained constant for up to 72 h at levels 2- to 3-fold greater than that achieved with free DOX (Harasym et al., 1997). Due to the increased leakage of DOX in the circulation, the drug presumably reaches normal tissues at a faster rate as

well (Mayer et al., 1989), although levels at very early times were not measured. When rates of lipid and DOX accumulation were compared, DOX delivered via rapid-release carriers reached peak levels by 1 h, whereas lipid levels did not peak until 48 h. With slow-release carriers, the initial rate of tumor accumulation is similar for lipid and drug (Bally et al., 1994; Goren et al., 1996), but at later times, tumor drug levels decrease upon drug release from the carrier and subsequent metabolism and redistribution (Goren et al., 1996). Drug can thus accumulate in tumor via several different mechanisms, although the primary mechanism for delivery by slow-release liposomal carriers is via extravasation of liposome plus drug through a discontinuous microvasculature. Studies of the effects of various factors (e.g., size, charge, tumor microenvironment, regulation by growth factors) on the permeability and distribution of liposomes within the tumor will help engineer liposomes for more effective delivery of their contents in the tumor. With the exception of one study (Yuan et al., 1994), few studies have considered the movement of liposomes through the tumor interstitium. The long distances and high interstitial pressure make this an obstacle that may prove as important as the permeability of the tumor vasculature.

IV. Efficacy of Liposomal Drugs in Animal Tumor Models

SSL DOX has been examined for antitumor efficacy in a variety of different tumor models, including a human lung tumor xenograft (Williams et al., 1993), human pancreatic carcinoma xenograft (Vaage et al., 1997), mouse lymphoma (Gabizon et al., 1996, 1997, 1998), rat brain sarcoma (Siegal et al., 1995; Gabizon et al., 1997), mouse colon carcinoma (Papahadjopoulos et al., 1991; Huang et al., 1992; Mayhew et al., 1992), prostatic tumor xenografts (Vaage et al., 1994a; Working et al., 1994), mouse mammary carcinomas (Mayer et al., 1990a; Forssen et al., 1992; Vaage et al., 1992), ovarian carcinoma xenograft (Vaage et al., 1993a), and an HER2-overexpressing human breast carcinoma xenograft (Park et al., 1997). The combination of a broad activity of DOX to a wide assortment of different cancers and the common mechanistic rationale for liposomal accumulation in solid tumors (see *IIIA. Mechanistic Rationale for Liposome Accumulation in Tumors: Enhanced Permeability and Retention Effect Phenomenon*) results in a drug formulation with substantial antitumor efficacy compared with the free drug and relatively independent of the type or location of the tumor. Even in a brain tumor model where the blood-brain barrier is thought to severely limit drug accumulation, SSL DOX showed a significant increase in mean survival times compared with free DOX (189% compared with 126%; Siegal et al., 1995; Gabizon et al., 1997). Efficacy results in a variety of different liposome formulations and tumor models are listed in Table 8. There are possible exceptions to these observations that may result in different liposome formulations being more suitable for the treatment of different cancers, depending on circumstances. These potential exceptions are described later.

In critical evaluation of previous studies using liposomal anthracyclines, one must be careful of comparisons drawn between CL and SSL formulations. All too often, the CL formulation being referred to is of a suboptimal formulation, containing either unsaturated lipids that allow the drug to leak rapidly from the carrier or negatively charged lipids that facilitate their clearance from the circulation. Under exceedingly complex conditions, such as in vivo drug delivery, where a variety of factors can influence pharmacokinetics, stability, extravasation into tumors, and clinical efficacy, it is best for comparisons to be drawn where a minimum number of variables, ideally only one, are altered at any one time.

A. Comparison of Efficacy for Sterically Stabilized and Conventional Liposomes

There are few studies that directly compare small neutral CLs and SSLs in therapeutic efficacy studies (Huang et al., 1992; Unezaki et al., 1995; Gabizon et al., 1996; Chang et al., 1997; Mayer et al., 1997; Parr et al., 1997). With the exception of one study that targets splenic and liver tumors (Chang et al., 1997), these comparisons are usually divided into one of two experimental designs. We examine the results and relevance of each of these experimental designs individually. In the first design, liposomes are injected at a dose of $\leq$10 mg/kg DOX and show significant improvement in therapeutic efficacy for SSL DOX compared with both free DOX and CL L-DOX (L-DOX; Table 8; Huang et al., 1992; Unezaki et al., 1995; Gabizon et al., 1996). Using a BALB/c mouse C26 colon carcinoma model, an increased life span (ILS) of 48.3% for SSL DOX was observed compared with 5.1% for DSPC/Chol liposomes and $-$4.2% for free DOX when administered as a single dose at 10 mg/kg (Huang et al., 1992). When SSL DOX or SSL-epirubicin was injected at either a single dose of 10 mg/kg DOX or three weekly doses of 6 or 9 mg/kg DOX, tumors regressed to a nonmeasurable size over time, whereas free drug administered at an identical dose and schedule only slightly delayed tumor growth compared with controls. This was similar to the first efficacy study completed with a Stealth® liposomal drug formulation showing increased therapeutic efficacy of SSL-epirubicin compared with free epirubicin (Papahadjopoulos et al., 1991). Epirubicin is a DOX analog that shows markedly reduced cardiac toxicity compared with DOX. In this study, both an increase in life span and an inhibition of tumor growth were noted with three weekly injections of SSL-epirubicin (6 mg/kg epirubicin), whereas free epirubicin had only minimal effects. Unfortunately, due to the significantly decreased ILS of the CL formulation, neither study examined the effect of CL DOX or

TABLE 8
Efficacy studies in animals

Animal Model	Formulation	Dose	ILS[a]	*p*
		mg/kg	%	
Fischer rats (fibrous histiocytoma)[e]	Doxil	8	65	<.0003
	Free DOX	8	35	<.05
	Doxil	5 × 3	89	<.00001
	Free DOX	5 × 3	23	<.05
$CD2F_1$ mice (P-1798 lymphosarcoma)[f]	DaunoXome	20	15	<.05
	Free daunorubicin	20	54	<.05
$B6C3F_1$ mice (MA16C mammary adenocarcinoma)[f]	DaunoXome	20	>217	<.05
	DaunoXome	2	100	<.05
	Free daunorubicin	20	94	
Nude mice (ovarian HEY cancer)[g]	Doxil	9	40	<.001
	Free DOX	9	−50	
Female BALB/c mice (C26 colon carcinoma)[h]	PEG-DSPE/HSPC/Chol (DOX)	10	48.3	
	DSPC/Chol (2:1)	10	5.1	NS
	Free DOX	10	−4.2	NS
BALB/c mice (J6456 lymphoma)	Doxil	10 (i.v.)	116	<.0001
	Free DOX	10 (i.v.)	21	<.0001
	Doxil	10 (i.p.)	60.5	<.0001
	Free DOX	10 (i.p.)	60.5	<.0001
Female BALB/c mice (C26 colon carcinoma)[j]	PEG-DSPE/HSPC/Chol (DOX)	10	−7	
	DSPC/Chol (1:1)	10	21	
	Free DOX	10	114	
BALB/c mice (M-109 carcinoma)[k]	PEG-DSPE/DSPC/Chol (DOX)	10	168	<.05
	PEG-DSPC/DPPC/Chol	10	118	
	DSPG/HSPC/Chol	10	168	<.1
	HSPC/Chol	10	94	
	Free DOX	10	97	
BALB/c mice (J6456 lymphoma)[k]	PEG-DSPE/DSPC/Chol (DOX)	15	144	<.01
	DSPG/HSPC/Chol	15	197	<.01
	Free DOX	15	19	
BALB/c mice (C26 colon carcinoma)[j]	PEG-DSPC/DSPC/Chol (epirubicin)	6 × 3	148[b]	
	Free epirubicin	6 × 3	20	
BDF1 mice (P388 lymphoma)[m]	DSPC/Chol (55:45) (VCR)	3	38	<.05
	SM/Chol (55:45)	3	214	<.05
	Free VCR	3	>445	<.05
B6D2F1 mice (P388 leukemia)[n] (i.p.)	PEG-DSPE/DSPC/Chol (VCR)	2	199	<.01[c]
	Free VCR	2	79	<.01[c]
BDF1 mice (L1210 tumors)[o]	DSPC/Chol (mitoxanthrone)	20	189	
	DMPC/Chol	20	>590	
	Free mitoxantrone	10[d]	98	
B6D2F1 mice (L1210 leukemia)[p] (i.v.)	PEG-DSPE/DSPC/Chol (ara-c)	50	138	
	PEG-DSPE/DSPC/Chol/SM	50	197	
	PG/DSPC/Chol	50	160	
	Free ara-C (24-h infusion)	50	90	

The composition, size, and drug/lipid ratio for DaunoXome and Doxil are listed in Table 1.
[a] ILS, mean survival: 100 * treated/control − 100.
[b] ILS was calculated only from mean survival time of mice dying before day 120 (there were 6 of 10 long-term survivors for >120 days).
[c] Between-treatment groups at same dose level; other *p* values are between treatment and control groups.
[d] The mean survival time for free mitoxantrone.
[e] Siegal et al. (1995).
[f] Forssen et al. (1992).
[g] Vaage et al. (1994b).
[h] Huang et al. (1992).
[i] Cabanes et al. (1998).
[j] Unezaki et al. (1995).
[k] Gabizon et al. (1996).
[l] Mayhew et al. (1992).
[m] Webb et al. (1995).
[n] Allen et al. (1995b).
[o] Lim et al. (1997).
[p] Allen et al. (1992).

CL-epirubicin on tumor growth. At the concentrations of DOX (6–9 mg/kg) and liposomes (50–75 mg/kg phospholipid) used in these studies, clearance rates are likely too rapid to observe a significant therapeutic result for CLs.

Two additional studies completed with a comparable experimental design showed similar results to those observed earlier (Unezaki et al., 1995; Gabizon et al., 1996). In an almost identical design (including the tumor and animal model), Unezaki et al. (1995) showed a 6-fold increase in the percent ILS for SSLs compared with CLs (Table 8). This correlated to a >3-fold decrease in tumor AUC levels for DOX when encapsulated in CLs (Table 6). Gabizon et al. (1996) completed one of the most thorough and careful studies of the effect of lipid composition on therapeutic efficacy. In this study, five different liposome compositions were evaluated for therapeutic efficacy and tumor accumulation in two different tumor models (J6456 lymphoma and M-109 carcinoma, a murine lung metastasis model). The number of variables was kept to a minimum to allow for careful com-

parisons, and even rates of accumulation in tumors were measured in some instances, rather than simply measuring DOX levels at single time points. At a single dose of 10 mg/kg, a linear correlation between circulation lifetimes and antitumor efficacy could not be found. Liposomes with similar but still reduced lifetimes (HSPI/HSPC/Chol, DSPG/HSPC/Chol, GM_1/HSPC/Chol) compared with PEG-DSPE/HSPC/Chol liposomes had a similar therapeutic efficacy. In one example, DSPG/DSPC/Chol liposomes were shown to have plasma levels at 24 h, approximately half that seen for PEG-DSPE/HSPC/Chol liposomes but an identical degree of accumulation in tumor and percent ILS. This was not shown to be an artifact of the type of tumor investigated, in that both the J6456 lymphoma and M-106 carcinoma gave similar results. Interestingly, the one liposome composition that showed significantly less efficacy was HSPC/Chol. Plasma levels were between one fourth and one half of the closest formulation and this translated into a reduced percent ILS (168 versus 94%) in both tumor models. For the conditions examined in this study, SSLs certainly showed a greater accumulation in tumors and an increase in therapeutic efficacy compared with neutral CLs (HSPC/Chol). However, the far more interesting result, obtained with other negatively charged formulations, demonstrated that longer circulation times do not necessitate greater tumor accumulation and efficacy.

In a second experimental design, liposomes were injected as a single dose of 20 to 55 mg/kg DOX and showed approximately equivalent therapeutic efficacy for SSL DOX and CL DOX (Table 8; Mayer et al., 1997; Parr et al., 1997). At these high doses, the circulation lifetimes for the CL formulation are markedly elevated due to a toxic effect on RES macrophages, and initial rates of tumor accumulation appear to be greater for CLs than for SSL. The rates of tumor accumulation favor the SSL formulation at later times, likely due to the more rapidly disappearing pool of CLs. In both of these studies, tumor growth rates were slowed to comparable extents with SSL DOX and CL DOX. There was no significant difference in either study. However, a serious question is raised in one of these studies due to the significant activity of free DOX (Mayer et al., 1997). This may be due to the nature of the tumor investigated: either methylcholanthrene-induced or "spontaneously" arising fibrosarcoma. In any case, there is no significant difference between free and liposome encapsulated DOX with one tumor, and free DOX actually appears more efficacious in the second tumor investigated. This stands in stark contrast to most other studies with L-DOX in which a significant therapeutic advantage is gained by liposome encapsulation. In the other study, L-DOX was used at a concentration (55 mg/kg), 5.5-fold greater than that used with previous studies with SSL DOX (Parr et al., 1997). This raises serious toxicological concerns, which are addressed in *VI. Toxicology of Liposomal Chemotherapy*. Even at these concentrations, tumor growth continued rapidly after a short delay. It is unlikely that the animals would tolerate multiple injections of the drug at these doses. The authors appear to make logical arguments as to why RES blockade and an increased permeability of the tumor microvasculature to CLs could give rise to similar therapeutic efficacy. However, the experiments in these two reports appear to be unconvincing because of the unrealistically high doses of drug being administered, and a more careful set of experiments on the effect of dose on tumor accumulation, toxicity, and therapeutic efficacy in already established tumor models may prove to be more persuasive. In addition, even at the elevated doses used in these studies, little information is given concerning the types and degree of severity of different toxicities. This appears to be an especially important concern considering the doses under investigation.

B. Model Dependency of Results

There are a number of characteristics of animal and tumor models, and the study design in general, that may influence the observed results for a given formulation in efficacy studies; these include such factors as the initial size of the tumor before the start of treatment, the growth rate of the tumor, the route of administration, the frequency of injection, and the tumor microenvironment. Investigators should be aware of how these factors potentially influence the observed efficacy of a particular liposomal drug, as well as comparisons between different formulations. Each of these characteristics is examined in detail here.

1. Initial Size of Tumor. The size of the tumor is an important determinant in its ability to be treated. As mentioned in *III. Accumulation of Liposomal Drugs in Tumors*, the tumor microvasculature varies depending on the size of the tumor. Vascular permeability has been shown to increase with increasing tumor size, and some very small lesions (<1–2 mm) appear to be avascular (Folkman, 1971, 1990; Blasberg et al., 1981; Zhang et al., 1992). Thus, some extremely small tumors may not be particularly amenable to treatment with liposomal drugs that require extravasation for activity. In other instances, small tumors may coopt already existing blood vessels (Holmgren et al., 1995; Pezzella et al., 1997; Holash et al., 1999). A more relevant problem occurs as tumors increase to very large sizes; the necrotic regions in the interior of large tumors have a reduced vascular density and an increased interstitial pressure compared with the surface of the tumor (Jain, 1987, 1990; Jain and Baxter, 1988; Baxter and Jain, 1990). The result is a reduced access of liposome-associated drug, which enters the tumor via extravasation, to certain areas of the tumor. Experiments can be effectively biased toward a favorable therapeutic outcome by choosing to start the drug administration at early times when the tumor size is small (<0.1 cm^3). Several studies

have shown a difference in the SSL DOX or CL DOX to free DOX efficacy depending on the day of treatment relative to tumor inoculation (Huang et al., 1992; Vaage et al., 1992; Cabanes et al., 1998). Of course, this applies to treatment with free drug as well; tumors treated early after tumor inoculation can be considerably easier to treat than those whose for which treatment was delayed (Huang et al., 1992). Recently, most experiments completed in our laboratory have used 0.20 to 0.25 cm^3 as the size at which treatments are begun.

2. Rapidly Growing versus Slowly Growing Tumors. Certain tumor models may give ambiguous results with long-circulating liposomes due to their rapid doubling times (Allen et al., 1992; Papahadjopoulos and Gabizon, 1995). Both L1210 and P388 leukemias in mice fit into this category. In these two models, the cells divide more rapidly than liposomes can distribute to tumors and release their contents. With fast growing tumors, liposomes that accumulate in tumors or release their contents more rapidly may be more efficacious. Thus, although DPPC/Chol or PEG-DSPE/DPPC/Chol liposomes may release their contents too rapidly to be effective against slower growing tumors, they may show greater efficacy than the slow-releasing HSPC/Chol liposomes in these tumor models. Allen et al. (1992) have shown that formulations with increased release rates of encapsulated ara-C were more efficacious in the treatment of mice injected with rapidly growing L1210 leukemia cells (Table 8). In addition, if CLs do accumulate more rapidly in tumors, they may have an advantage over SSLs, even if the long-term accumulation is not as great. As mentioned previously, the rate of liposome accumulation in tumors remains a point of controversy and must be more thoroughly studied. Of course, the rates of tumor drug accumulation and drug release rates from liposomes must be of a similar magnitude to be the most effective in the delivery of bioavailable drug to tumors. If liposomes release most of the drug before reaching the tumor or are taken up so rapidly by the RES that they cannot accumulate in tumors to a significant extent, then the effective concentration of bioavailable drug in the tumor will still be less than that for long-circulating liposomes. Most solid tumors targeted in animal studies grow at a sufficiently slow rate, as to be compatible with long-circulating liposomes.

The difference in tumor doubling times between human and animal tumor models may also play a role in the effectiveness of a particular type of liposomal treatment. The animal tumor models described in many of these studies have doubling times on the order of days to a few weeks. Most human tumors have doubling times on the order of weeks to months, a substantial increase compared with that seen in animals. In slowly growing tumors, small differences in the rate liposomes accumulate in tumors, or the rate at which the drug becomes bioavailable (i.e., is released from the carrier) will have less impact on efficacy than in rapidly growing tumors, where the overall flux of bioavailable drug through the tumor is more likely to determine treatment success. The move from animals to humans should favor SSLs, where liposomes continue to accumulate in tumors for days after administration.

3. Route of Administration. The route of administration is another important variable when considering the relative therapeutic enhancement provided by liposomes in the treatment of cancer. The i.v. route is the commonly used route of administration for liposomal drugs due primarily to its ability to reach distant sites of metastasis. Because the vasculature of even tumor metastasis requires angiogenesis and increased vascular permeability to obtain the nutrients required for its rapid growth, delivery via the i.v. route allows the drug to be simultaneously targeted to all sites of primary growth or metastases. Delivery via other routes may reduce the amount of drug that effectively reaches the tumor and thus decrease the efficacy of the drug. In a mouse J6456 lymphoma model, SSL DOX injected i.v. was shown to increase the ILS from 121 to 215 ($p < .0001$) compared with free DOX at 10 mg/kg (Table 8; Cabanes et al., 1998). When administered by i.p. injection, the life spans were identical (ILS = 60.5%) for free and L-DOX, showing that a considerable difference in effectiveness does exist depending on the site of administration.

4. Frequency of Injection. The frequency of drug injection is also likely to have an effect on the therapeutic response. CLs require high doses of both lipid (>100 mg/kg) and drug (>20 mg/kg) to obtain comparable tumor levels of drug to SSL DOX (see *IIIB. Rate and Extent of Accumulation in Tumors*; Mayer et al., 1997; Parr et al., 1997). At these high doses, repeated injections may not be possible due to nonspecific toxicities. In addition to the toxicities associated with the drug, Allen et al. (1984) have shown that multiple injections of free liposomes at high doses also cause significant toxicity to liver and spleen (see *VIA. Tolerability of Liposome Components*). The dose independence of SSL DOX allows liposomes to be administered at low doses on a schedule that varies from once a week to once every 4 weeks. This is likely necessary to keep tumor drug levels high and thus maintain a greater efficacy. If CLs are found to have similar efficacy at a single high dose, then the next step will be to show that this similarity in efficacy can be maintained after multiple injections without compromising the reduced toxicity of the drug.

5. Environment of Tumor. The site of tumor implantation is also important in determination of the relative efficacy of a liposomal drug formulation. Tumors vary in permeability, vascular density, and response to local permeability or growth factors depending on the microenvironment of the tumor (Dellian et al., 1996; Fukumura et al., 1997; Hobbs et al., 1998). Tumors implanted s.c. have different properties from those implanted in the liver or in the brain. Tumors found in the liver and

spleen may be more susceptible to drug delivered by CLs than tumors in other areas of the body, due to the ability of CLs to localize rapidly and preferentially in these organs. Drug released from macrophages may kill neighboring tumor cells through the bystander effect (Storm et al., 1988). This underscores the question as to why more studies have not been completed with liver metastatic models. Early studies completed with DOX loaded in PG- or PS-containing liposomes demonstrated an enhanced activity toward liver metastasis of colon carcinomas (Mayhew et al., 1987) or lymphomas (Gabizon et al., 1993) compared with free DOX. These, or similar, liposome formulations were ineffective against a variety of cancers located elsewhere in the body (Gabizon et al., 1990). The close proximity of liver metastasis to Kupffer cells responsible for liposomal drug uptake may make tumor models more sensitive to treatment with liposomal drug therapy and alter the characteristics necessary for effective treatment.

Allen et al. (1992) showed that mice injected i.v. with L1210 leukemia cells were treated more effectively after the encapsulation of ara-C in liposomes than by a 24-h infusion of free ara-C, presumably because liposomes can more efficiently deliver the drug to liver or splenic tumors. In the same study, liposomes that released ara-C at a faster rate were shown to have the greatest activity (Table 8). This same group used liposomal VCR to show that depending on the route of tumor implantation for L1210 leukemic cells, drugs may benefit from either a more rapid or a slower release from the liposomal carrier (Allen et al., 1995b). These authors concluded that peritoneal or s.c. tumors may be more amenable to slow-release systems, whereas intravascular cancers are better treated with rapid-release systems. Another rather elegant study used liposomal mitoxantrone encapsulated in different liposome formulations to show that liposomes that rapidly release their contents may be more efficacious than slow-release formulations in two such tumor models (Lim et al., 1997). L1210 and P388 cells seed preferentially in the liver and spleen when injected i.v. (Lim et al., 1997). In this study, mitoxantrone delivered via DMPC/Chol liposomes (rapid-release liposomes) were considerably more effective than when delivered via DSPC/Chol liposomes (Table 8; >590 versus 189% ILS), suggesting the rapid leakage of drug from the former carrier may facilitate the cytoxic activity of the drug in this model. In addition, CLs (DSPC/Chol) were shown to have a similar percent ILS compared with SSL-mitoxantrone at both 10 and 20 mg/kg (Table 8; Chang et al., 1997). Although SSL-mitoxantrone may distribute to the tumors themselves to a greater extent, the high concentration of mitoxantrone in the liver and spleen, due to its rapid uptake by RES macrophages in these organs, may provide higher overall concentrations of bioavailable drug after its release from these macrophages. Unlike DOX, mitoxantrone was unable to increase the circulation lifetime by poisoning RES macrophages. The studies suggest that distribution to the liver and spleen may favor a good therapeutic response with CLs programmed to release the encapsulated drug at a faster rate than experienced with solid DSPC/Chol or PEG-DSPE/DSPC/Chol liposomes. Although these experiments show that proximity of tumors to RES macrophages may alter the characteristics needed to engineer effective liposomal drug delivery vehicles for their treatment, it should be noted that both L1210 and P388 tumors are fast growing tumors that likely favor rapid drug release for the reasons mentioned earlier. It would be interesting for the authors to repeat these experiments with slower growing tumors to see whether a more general relationship truly exists between tumor environment and drug-release characteristics.

C. Efficacy with Nonanthracyclines

There have been several in vivo therapeutic efficacy studies completed with drugs other than anthracyclines. SSL-cisplatin (SPI-77) is presently being developed by Alza Corporation (Palo Alto, CA; formerly Sequus Pharmaceuticals, Inc., Menlo Park, CA). Although details are limited, SSL-cisplatin proved more efficacious than free cisplatin in both Lewis lung carcinoma and C26 colon carcinoma tumor models (Working et al., 1998; Newman et al., 1999), delaying tumor growth in one model by >30 days compared with 3.7 for free cisplatin. In another study, SSLs were used to deliver ara-C in a murine L1210/C2 leukemia model (Allen et al., 1992). Encapsulation in SSLs increased the percent ILS from 90 to 197%. The liposomes were shown to act as a slow-release depot for drug, and the increased therapeutic efficacy was thought to result primarily from this effect and not preferential accumulation in tumors. These liposomes were not optimized for long circulation and high tumor accumulation because the liposomes were prepared by reversed-phase evaporation followed by extrusion through 0.4-μm-pore filters, giving liposomes in excess of 400 nm.

Several studies have been completed with liposomal paclitaxel (Sharma et al., 1995, 1996, 1997). In two of these studies, liposomal paclitaxel showed significant activity against human ovarian tumor xenografts, inhibiting tumor growth (Sharma et al., 1995, 1997). The liposomes in these studies incorporated paclitaxel into liposomes at low drug/lipid ratios (1:33), much lower than that used for DOX (see Table 1), likely a result of the drug being carried in the liposomal membrane and not encapsulated within its internal aqueous space, as is DOX. This is consistent with the physicochemical properties of the drugs, where hydrophobic drugs such as paclitaxel would be expected to reside in the hydrophobic membrane core and amphipathic drugs, such as DOX, can be loaded by remote-loading techniques (see *VIIA1. Drug-Loading Methods*) to high concentrations in the encapsulated aqueous space. The pharmacokinetic pa-

rameter associated with the drug were similar for liposomal paclitaxel and paclitaxel formulated with Cremophor EL, suggesting that liposomes in this formulation are simply acting as a drug-solubilizing agent (Sharma et al., 1997), and the drug rapidly redistributes to other hydrophobic sites after administration. The high toxicity of the Cremophor EL vehicle makes delivery by liposomal solubilization therapeutically beneficial, due to the low toxicity of the liposomal carrier (see *VIA. Tolerability of Liposome Components*). However, because this formulation acts as an extremely rapid-release liposomal carrier, it differs from slow-release systems, which selectively accumulate in tumors and release their contents on a more compatible time scale. A lipophilic cisplatin derivative, *cis*-bisneodecanoato-*trans*-*R*,*R*-1,2-diaminocylcohexaneplatinum(II), has also shown some promise when incorporated into liposomal membranes (Mori et al., 1996). This prodrug reverts to the active drug after hydrolysis.

Finally, liposomal VCR has been the most thoroughly studied nonanthracycline liposome formulation in vivo. VCR is an alkaloid derived from *Vinca rosea* that has been used clinically for the treatment of various types of cancer (Carter and Livingston, 1976). Like other *Vinca* alkaloids, VCR exerts its antitumor activity by inhibiting cell division via interactions with tubulin (Owellen et al., 1976). The major dose-limiting toxicities of VCR is a peripheral neurotoxicity (Rowinsky and Donehower, 1996). VCR exhibits low solubility in aqueous solution at physiological pH and relatively high permeability to membranes. Due to physicochemical similarities with DOX, methods of drug loading in liposomes developed for DOX could be efficiently used for VCR; this is discussed in more detail in *VIIA1. Drug-Loading Methods*.

For CL formulations, the exchange of DSPC (T_m > 37°C) for eggPC ($T_m \ll 37$°C) increased the circulation lifetime of VCR by >100% (Mayer et al., 1990b, 1993). Despite this advance, no significant difference in the toxicity profile were observed compared with free VCR in dogs, although a moderate reduction in toxicity could be observed in mice (Kanter et al., 1994). However, the drawback of liposomal drug retention was still to be overcome because 85 to 90% of encapsulated VCR leaked from DSPC/Chol liposomes within 24 h of i.v. administration (Boman et al., 1994). Webb et al. (1995) showed that drug retention was increased after the substitution of SM for DSPC, and this translated into a significant improvement in efficacy in BDF1 mice bearing P388 tumors (214 versus 38% ILS for SM- and DSPC-containing liposomes, respectively). This demonstrates the importance of maintaining a stable formulation for the effective use of liposomes as a drug delivery system and that a particular lipid composition is not necessarily the best for all drugs, even if both are amphipathic in nature. Each drug must be considered individually.

SSL formulations of VCR in which liposomes were coated with GM_1 showed highly efficient cures of mice with P388 leukemia (Boman et al., 1994). When PEG was used as the stabilizing agent, liposomal VCR showed efficient antitumoral activity in s.c. and i.p. solid tumors but did not improve efficacy on rapidly growing i.v. disseminated leukemias (Allen et al., 1995b). This study also did not find a significant difference in the LD_{50} of SSLs and free VCR in mice, with both having an LD_{50} of ~2.5 mg/kg (Allen et al., 1995b). In this study, pharmacokinetic studies comparing SL- and CL-VCRs were performed using EPG/HSPC/Chol liposomes as a CL formulation. Although similar in surface charge to the SSL formulation, the presence of the exposed negative charge in EPG results in a relatively rapid clearance of the CL formulation from the circulation, essentially accentuating the differences between CLs and SSLs. Other studies have used a similar CL control with studies of L-DOX delivery (Mayhew et al., 1992; Vaage et al., 1992; Williams et al., 1993; Sakakibara et al., 1996). In our opinion, the use of small neutral CL formulations as a control when comparing SSL and CL formulations may be more accurate and informative as to the extent of the differences between optimized formulations of both SSLs and CLs. Finally, combination therapy, using SSL VCR and DOX, gave highly efficient stop growth and disappearance of mammary carcinoma MC2 bearing mice at doses for which no toxic systemic side effects could be detected (three weekly injections of 1.3 and 6 mg/kg for liposomal VCR and DOX, respectively; Vaage et al., 1993b).

D. Multidrug Resistance

Multidrug resistance can severely limit the effectiveness of some types of chemotherapy. Although drug resistance can take on many forms, one of the most common comes in the form of the multidrug resistance transporter, a membrane-spanning ATPase located in the plasma membrane and responsible for the efflux of positively charged amphipathic drugs from the cell (Endicott and Ling, 1989; Pastan and Gottesman, 1991; Gottesman and Pastan, 1993). Overexpression of P-glycoprotein (Pgp170) in tumor cells can lead to a marked decrease in sensitivity to drugs such as DOX. The delivery of L-DOX has resulted in the effective treatment of a number of chemotherapy refractory cancers both in animal models and in the clinic (Treat et al., 1990; Vaage et al., 1994b; Muggia et al., 1997; Northfelt et al., 1997). This has raised some questions as to whether L-DOX is able to sensitize tumor cells and thus partially reverse multidrug resistance.

Results in cell culture suggested that drug resistance could be partially reversed by treatment with L-DOX, although these cells were still significantly less sensitive than non-drug-resistant cell lines (Richardson and Ryman, 1982; Thierry et al., 1989; Rahman et al., 1992). The mechanism responsible for liposome-mediated partial reversal of drug resistance is not well understood. Several of the formulations used in these studies contain negatively charged phospholipid components, such as

phosphatidylserine (Fan et al., 1990) or cardiolipin (Thierry et al., 1989; Oudard et al., 1991; Rahman et al., 1992), which may act to directly regulate the P-glycoprotein transporter. Alternatively, they may act to provide sustained high levels of drug to the resistant cells over long periods of time (Allen, 1998), or if endocytosed, they may deliver the drug internally where it doesn't immediately reach the P-glycoprotein transporters located in the plasma membrane (Mickisch et al., 1992). Apart from cell sensitization, liposomal drug delivery may help overcome a broader range of drug resistance due to favorable pharmacokinetics. Thus, the increased response rates in these refractory patients may have to do with the increased concentration of drug that accumulates in the tumor after treatment with L-DOX. In any event, although the fact that L-DOX appears to be more effective against refractory patients is an encouraging observation, the likelihood that DOX delivered via liposomes will completely reverse multidrug resistance is low. Thus, to more effectively treat patients resistant to a particular type of chemotherapy, it will be important to combine L-DOX with other presently used chemotherapeutic agents or develop additional liposomal chemotherapeutic agents, with nonoverlapping mechanisms of drug resistance.

V. Clinical Efficacy of Liposomal Anthracyclines

There are three forms of L-DOX or daunorubicin being manufactured by different pharmaceutical companies. The properties of these formulations are given in Table 1. Doxil and DaunoXome have been approved for the treatment of AIDS-related Kaposi's sarcoma and are being evaluated in clinical trials for the treatment of a variety of cancers (Eckardt et al., 1994; Gill et al., 1995; Muggia et al., 1997; Ranson et al., 1997; Martin, 1998; Northfelt et al., 1998; Schmidt et al., 1998). The Liposome Company, Inc. has recently completed several large phase II and phase III clinical trials using EVACET (also known as TLC D-99) for the treatment of metastatic breast cancer and is now awaiting approval for the drug by the Food and Drug Administration (Harris et al., 1998; Swenson et al., 1998; Valero et al., 1999). The data obtained from trials thus far suggest that all three liposomal drugs offer a significant therapeutic benefit compared with the free drug and often compared with current chemotherapy combinations indicated for the studied form of cancer. Liposomal drugs can be therapeutically beneficial based on their ability to decrease nonspecific toxicities associated with the drug, a process referred to as toxicity buffering, or by being more efficacious against a specific type of cancer, increasing the response frequency, average time to relapse, or response duration. DaunoXome and Doxil have been shown to offer similar or greater efficacy, and decreased levels of most toxicities (see *VI. Toxicology of Liposomal Chemotherapy*; Table 9) compared with free DOX and standard chemotherapy regimens (Gabizon et al., 1994; Gill et al., 1995, 1996; Muggia et al., 1997; Ranson et al., 1997; Martin, 1998; Northfelt et al., 1998; Stewart et al., 1998). EVACET was shown to decrease most toxicities and has a similar response frequency to DOX alone (Batist et al., 1998; Harris et al., 1998).

This review focuses primarily on PC/Chol CLs and SSLs. There have been several earlier clinical studies with alternative formulations, mostly containing small quantities of negatively charged lipids, but due to their unproven clinical utility they are not discussed further in this review. The reader is referred to the following references for information on these studies (Gabizon et al., 1989, 1991; Treat et al., 1990; Rahman et al., 1992; Gabizon, 1998). In addition, efficacy can be dependent on a variety of different patient characteristics; these include such characteristics as sex, age, prior chemotherapy treatments, degree of disease severity, presence of metastatic disease, and overall performance status. The reader is encouraged to return to the original citations to obtain information concerning these characteristics because a detailed evaluation of the complete clinical findings is beyond the scope of this review.

A. AIDS-Related Kaposi's Sarcoma

Kaposi's sarcoma is the most common neoplasm associated with AIDS (Northfelt, 1994). It is characterized by painful and disfiguring cutaneous lesions that can also have tumor-associated lymphedema. Some patients have visceral involvement, including gastrointestinal and pulmonary nodules. Single-agent standard chemotherapy is relatively ineffective. Until recently, combination regimens, including bleomycin/VCR (BV) or DOX/bleomycin/VCR (ABV), were most commonly used as a front-line defense (Gill et al., 1990, 1994). Both liposomal daunorubicin and SSL DOX have shown significant activity against Kaposi's sarcoma in a number of phase II and III clinical trials (Presant et al., 1993; Simpson et al., 1993; Gill et al., 1995, 1996; Harrison et al., 1995; Girard et al., 1996; Amantea et al., 1997; Coukell and Spencer, 1997; Northfelt et al., 1997, 1998; Stewart et al., 1998). Response rates have varied from 25 to 73.5% depending on patient characteristics and trial design. Many of the results from these trials are listed in Table 9.

Recently, Doxil (20 mg/m^2) was shown to compare very favorably with either the ABV (20 mg/m^2:10 mg/m^2:1 mg) or BV (15 IU/m^2:2 mg) regimens (Northfelt et al., 1998; Stewart et al., 1998). The overall response rate in the ABV comparison was 45.9% for Doxil and 24.8% for the ABV arm (Northfelt et al., 1998). Doxil showed an overall response rate of 58.7% compared with 23.3% for the BV arm (Stewart et al., 1998). In both of these studies, the duration of response was similar for both arms of the study. In addition to the superiority in response rate achieved with Doxil, both studies reported a significant decrease in certain toxicities and greater

TABLE 9
Phase II and III clinical studies with L-DOX

Cancer[a]	Liposome Formulation[b]	Dose	Schedule	Total Responses	Complete Response	Response Duration
		mg/m²		*%*		*days*
Kaposi's sarcoma (n = 16)[e]	Doxil	20	×3 wk	75		98
Kaposi's sarcoma (n = 24)[f] (patients failed standard chemotherapy)	DaunoXome	40	×2 wk	54.2	8.3	84
Kaposi's sarcoma (n = 22)[g]	DaunoXome	50–60	×2 wk	55	5	
Kaposi's sarcoma (n = 34)[h]	Doxil	20	×3 wk	73.5	5.8	63
Kaposi's sarcoma (n = 29)[i]	Free DOX	20	×2 wk	48	3	105
Kaposi's sarcoma (n = 116)[j]	DaunoXome	40	×2 wk	25	2.5	175
	ABV	10, 15 U, 1 mg	×2 wk	28	0.9	168
Kaposi's sarcoma (n = 30)[k]	DaunoXome	40	×2 wk	73		153
Kaposi's sarcoma (n = 29)[l]	DaunoXome	40	×2 wk	45		70
Kaposi's sarcoma (n = 53)[m] (patients failed standard chemotherapy)	Doxil	20	×3 wk	36	1.8	128
Ovarian carcinoma (n = 35)[c, n]	Doxil	40–50	×3 wk	25.7	2.9	180
Metastatic breast carcinoma (n = 64)[o]	Doxil	45–60	3–4 wk	31	6.3	270
Kaposi's sarcoma (n = 40)[p]	Doxil	20	×3 wk	70		
Kaposi's sarcoma (n = 121)[q]	Doxil	20	×3 wk	58.7	5.8	160.4
	BV	15 IU/m², 2 mg	×3 wk	23.3	0.8	156.7
Kaposi's sarcoma (n = 133)[r]	Doxil	20	×2 wk	45.9	0.8	90
	ABV	20, 10, 1 mg	×2 wk	24.8		92
Metastatic breast carcinoma (n = 69)[s]	TLC D-99	75	×3 wk	33	3	
	Free DOX	75	×3 wk	28	1	
Metastatic breast carcinoma (n = 69)[t]	TLC D-99/ CPA/5-FU[c]	60, 500, 500	×3 wk[d]	68	5	

CPA, cyclophosphamide; 5-FU, 5-fluorouracil.
[a] Values are number of accessable patients.
[b] The composition, size, and drug/lipid ratio for DaunoXome and Doxil are listed in Table 1.
[c] Patients failed to respond to platinum- and paclitaxel-based regimens.
[d] Both CPA and TLC D-99 were administered on day 1, and 5-FU was administered on days 1 and 8.
[e] Simpson et al. (1993).
[f] Presant et al. (1993).
[g] Gill et al. (1995).
[h] Harrison et al. (1995).
[i] Gill et al. (1991).
[j] Gill et al. (1996).
[k] Girard et al. (1996).
[l] Uthayakumar et al. (1996).
[m] Northfelt et al. (1997).
[n] Muggia et al. (1997).
[o] Ranson et al. (1997).
[p] Amantea et al. (1997).
[q] Stewart et al. (1998).
[r] Northfelt et al. (1998).
[s] Harris et al. (1998).
[t] Valero et al. (1999).

patient compliance with the liposomal drug. Similar to patients receiving daunorubicin (Gill et al., 1996), patients receiving Doxil developed more opportunistic infections than those receiving the standard chemotherapy regimens (Stewart et al., 1998). The toxicological advantages are described in more detail in *VI. Toxicology of Liposomal Chemotherapy*.

DaunoXome was also compared with the ABV regimen in a large randomized trial (232 patients; Gill et al., 1996). DaunoXome (40 mg/m^2) was found to have an overall response rate of 25 compared with 28% for the ABV arm (10 mg/m^2:15 U:1 mg) and an almost identical response duration (175 versus 168 days). Although DaunoXome did not appear to have any advantage over the ABV regimen in terms of response rate, patients receiving DaunoXome experienced less alopecia (8 versus 36%) and neuropathy (13 versus 41%) but a slightly greater incidence of opportunistic infections. Other differences in toxicities observed were not statistically significant.

An indirect comparison of Doxil and DaunoXome suggests that Doxil is significantly more active against Kaposi's sarcoma than DaunoXome. Although DaunoXome is comparable in response rate to the ABV regimen, Doxil shows a considerable increase in response rate compared with both ABV and BV regimens. The response duration was shorter for the Doxil study completed by Stewart et al. (1998). However, the duration of response was likely underestimated due to the inclusion of stable disease as an endpoint for response and the restriction in this study of a maximum number of six cycles of drug therapy (Bennett et al., 1998). Toxicities also appeared to favor Doxil over DaunoXome. This is not surprising, considering the DaunoXome dose was

twice that of Doxil (40 versus 20 mg/m^2) and injections (every 2 weeks versus every 3 weeks) of daunorubicin were given at a higher frequency. In the treatment of Kaposi's sarcoma at least, SSLs appear to be far more efficient drug-delivery vehicles than CLs. Although DOX and DaunoXome are very similar in mechanism of action, pharmacokinetic parameters, and toxicological profiles, the difference in the encapsulated drug precludes us from making any definitive statements concerning the superiority of SSLs based on these observations. In addition, the weak immune system and increased susceptibility to opportunistic infections of AIDS patients prevent escalation of the daunorubicin dose to dosages that may allow for longer circulation lifetimes of CLs.

B. Treatment of Breast and Ovarian Carcinomas

L-DOX was suggested to have greater activity against breast and ovarian cancers, which are typically only moderately sensitive to DOX, due to the enhanced tumor accumulation of the drug. In patients with advanced ovarian cancer, who were refractory toward paclitaxel- and platinum-based regimens, Doxil was shown to have a response rate of 25.7% and a response duration of 180 days (Table 9; Muggia et al., 1997). This favorable response rate was significantly greater than that for free DOX in similar patients (<10%; Young et al., 1981), and there were fewer problems with patient compliance due to reduced toxicities (Alberts and Garcia, 1997).

Two studies have been completed with SSL DOX (Doxil) and CL DOX (TLC D-99) in metastatic breast cancer patients (Table 9; Ranson et al., 1997; Harris et al., 1998). Response rates were similar in the two studies (31% for Doxil and 33% for TLC D-99). The response rate of free DOX was 29% in the randomized phase III study comparing TLC D-99 and free DOX (both at 75 mg/m^2). This response rate for free DOX was similar to that described previously in similar patients (Young et al., 1981). It will be interesting to do a more detailed comparison of Doxil and TLC D-99 when the full results from the clinical trials with TLC D-99 are published. Pharmacokinetic and tumor accumulation considerations would theoretically favor Doxil. However, TLC D-99 is administered at a dose of 75 mg/m^2 compared with 45 mg/m^2 for Doxil (both are administered every 3 weeks). Thus, the higher dose and increased rate of drug release from the carrier at the tumor may contribute to its similar activity. The principal reason for the decreased dose in the case of Doxil is the high incidence of hand and foot (H-F) syndrome associated with Doxil at elevated doses (60 mg/m^2). In both studies with ovarian and breast cancers, H-F syndrome was dose limiting (Muggia et al., 1997; Ranson et al., 1997). H-F syndrome is characterized by dermal lesions on both the palms of the hand and soles of the feet and is also found in patients receiving prolonged infusions of some chemotherapeutic agents. This toxicity is described in more detail in *VI. Toxicology of Liposomal Chemotherapy*. If the severity of H-F syndrome can be controlled by means other than dose reduction, then the administration of a similar dose will almost certainly give rise to an enhanced therapeutic effect. Mucositis, another significant toxicity in patients treated with Doxil, causing dose modification in some patients (Muggia et al., 1997; Ranson et al., 1997). The plasma AUC of TLC D-99 at elevated doses (Cowens et al., 1993; Embree et al., 1993) is far below the plasma AUC of Doxil at 25 or 50 mg/kg DOX (Table 4; Gabizon et al., 1994). These results emphasize the fact that the long circulating property of SSLs is not the only factor responsible for increased levels of efficacy with liposomal drugs. A mechanism by which DOX is at least partially released from the liposome in the circulation, avoiding the high peak levels of drug responsible for some types of toxicities, is most likely responsible for the increased therapeutic effect of TLC D-99. Thus, the altered toxicity profile of TLC D-99 allows the dose to be escalated to a point at which the efficacy is comparable to Doxil. The long-term effects of this dose escalation on the cumulative cardiotoxicity of DOX, compared with Doxil, are unknown. However, recent results did show a significant reduction in the cardiotoxicity when TLC D-99 was compared with free DOX (Batist et al., 1998). The reader is also referred to other reviews on the clinical activity of both Doxil (Gabizon, 1994, 1998; Coukell and Spencer, 1997; Muggia, 1997; Martin, 1998) and DaunoXome (Forssen and Ross, 1994; Schmidt et al., 1998) for additional analysis of the clinical data.

VI. Toxicology of Liposomal Chemotherapy

A. Tolerability of Liposome Components

"Empty" CLs or SSLs are usually considered nontoxic unless administered at very high doses (Storm et al., 1993; Working and Dayan, 1996). This is one of the characteristics that makes them attractive as a drug delivery vehicle and is not surprising because they are typically composed of natural lipids and small amounts of well-tolerated synthetic stabilizers (PEG-DSPE). At very high doses (multiple injections at a dose of ≥100 mg/kg lipid), liposomes have been shown to result in an impairment of RES function, hepatomegaly, granulomas, and splenomegaly (Allen et al., 1984, 1987; Allen and Smuckler, 1985; Storm et al., 1993). However, these effects are usually considered irrelevant due to the dose-limiting effects of the encapsulated drug. PEG is considered nontoxic at the degree of polymerization (1900–5000 Da) used to prepare SSLs and is excreted unmetabolized in the urine (Carpenter et al., 1971). Toxicity studies completed with PEG-DSPE micelles at a concentration 30-fold greater than that applied in a standard dose of SSL DOX demonstrated no deaths or clinical signs of toxicity (Working and Dayan, 1996). In addition, the various types of toxicities observed with SSL DOX treatment in animals are consistent with

those for free DOX, although at significantly reduced levels (Working and Dayan, 1996). Although for SSL DOX these results suggest that the lipid components have little if any effect on the overall toxicity profile, the picture for CLs is less clear. SSL DOX is typically administered at a total lipid dose of 8 to 54 mg/kg (1–6 mg/kg DOX) in rats or mice, with subsequent injections occurring between 3 days and 4 weeks. However, using CL a number of recent studies have used a single bolus injection of 100 to 300 mg/kg total lipid and 20 to 55 mg/kg DOX (Mayer et al., 1989, 1997; Parr et al., 1997). One of these studies showed minimal improvements in therapeutic efficacy over free DOX (Mayer et al., 1997). If multiple injections are to be used, then the toxicity of the lipid component may eventually become important; this will require further study.

In addition, an increasing lipid dose has been shown to deplete plasma of various proteins (Senior, 1987; Oja et al., 1996). Both the quantities and types of protein bound to liposomes are dependent on the lipid composition (Senior, 1987; Oja et al., 1996). Although the identity and significance of all the depleted proteins are unclear, it is possible that their loss will result in a disruption of normal homeostasis. Although toxicities related specifically to high lipid doses might prove to be relevant in some situations, in most instances the toxicity of the encapsulated drug is considered to be far more limiting.

B. Toxicities Associated with Free Drug

For the treatment of cancer, liposomal drug delivery has primarily involved the use of anthracyclines such as DOX or daunorubicin (Gabizon, 1994; Papahadjopoulos and Gabizon, 1995; Martin, 1998) or *Vinca* alkaloids such as VCR (Vaage et al., 1993b; Allen et al., 1995b; Webb et al., 1995). A few studies have chosen alternative drugs such as paclitaxel (Sharma et al., 1996, 1997), ara-C (Allen et al., 1992), methotrexate (Matthay et al., 1989; Jones and Hudson, 1993), or cisplatin derivatives (Perez-Soler et al., 1990; Mori et al., 1996), but preclinical toxicological results with these drugs are limited at the present time. Because the toxicity profile of a liposomal drug is primarily dependent on the encapsulated drug rather than the lipids, it is first important to understand the toxicities associated with the free drug. With free DOX, myelosuppression is considered dose limiting (Legha et al., 1987; Speth et al., 1988; Doroshaw, 1996). However, the therapy-limiting toxicity is considered to be cardiotoxicity and results after a high cumulative dose of the drug (Von Hoff et al., 1979; Doroshaw, 1996). High peak levels of DOX in the plasma have been shown to correlate with an increased risk of cardiac toxicity. When delivered by bolus injection, the most commonly used total cumulative dose of DOX is 450 to 500 mg/m^2, where the risk of cardiac toxicity is between 1 and 10% (Doroshaw, 1996). There is significant evidence that DOX delivered by continuous infusion displays similar efficacy but reduced toxicity, with cumulative doses up to 1100 mg/m^2 delivered without signs of cardiac toxicity (Legha et al., 1987; Hortobagyi et al., 1989). Although drug-induced congestive heart failure is the most significant concern due to its very poor prognosis, it is lethal in 60% of patients (Von Hoff et al., 1979); there are several other important toxicities associated with anthracyclines.

DOX-induced myelosuppression and alopecia are also delayed toxicities but are independent of the rate of drug administration (Speth et al., 1988; Doroshaw, 1996). Recent work has shown that myelosuppression can be partially combated with the use of colony-stimulating factors (granulocyte-CSF and granulocyte-macrophage-CSF), which stimulate activation and proliferation of hematopoietic cells (Vose and Armitage, 1995; Petros and Peters, 1996; Henry, 1997; Lieschke et al., 1997; Nemunaitis, 1997; Clemons et al., 1998). These growth factors are currently being used to allow dose intensification of conventional chemotherapy by effectively reducing one of the most common dose-limiting toxicities. Another toxicity associated with anthracyclines is a severe necrosis of the skin adjacent to the site of injection due to injection related drug extravasation (Von Hoff et al., 1979; Doroshaw, 1996). The resulting lesions are difficult to treat, and extreme care should be taken to prevent infection at these sites. Nausea, vomiting, and mucositis, including gastrointestinal toxicity and stomatitis, are additional toxicities resulting from chemotherapy with anthracyclines (Speth et al., 1988; Doroshaw, 1996). Finally, H-F syndrome, which is characterized by severe dermal lesions on the soles of the feet and the palms of the hand, is seen in patients receiving long-term continuous infusions of free DOX (Lokich and Moore, 1984; Vogelzang and Ratain, 1985). As discussed in *Effect of Liposome Encapsulation on Toxicity Profile*, although encapsulation of anthracyclines in liposomes does not alter the types of toxicities observed, the severity can be significantly reduced due to the resulting alterations in the pharmacokinetics and tissue distribution of the drug. In addition to these alterations, toxicity buffering is also a result of the relatively slow release of the DOX from the liposome, giving rise to relatively low peak levels of the free drug in the circulation.

C. Effect of Liposome Encapsulation on Toxicity Profile

1. Cardiotoxicity. The toxicity profile for L-DOX is altered due to the changes in pharmacokinetics described previously in *II. Pharmacokinetics and Biodistribution of Liposomes and Liposomal Drug*. Drug encapsulation in either SSLs or CLs eliminated or significantly reduced the amount of cardiotoxicity compared with the free drug (Table 10; Olson et al., 1982; Herman et al., 1983; Balazsovits et al., 1989; Working and Dayan, 1996; Working et al., 1999). This is thought to be due to the inability of liposomes to cross the endothelial cell barrier in the heart and the low bioavailabil-

TABLE 10
Toxicities associated with free and L-DOX

Toxicity	Effect of Liposome Encapsulation
Cardiac toxicity	Reduced or not observed
Myelosuppression	Reduced (to a greater extent with SSLs)
Mucositis	Slightly increased with Doxil
Alopecia	Reduced or not observed
Severe local tissue necrosis after drug extravasation	Reduced
Nausea and vomiting	Reduced or not observed
H-F syndrome	Observed with Doxil or with continuous infusion of free DOX

ity of the free drug due to its encapsulation in liposomes (Gabizon, 1994, 1997). Indeed, in tissue sections of cardiac muscle, liposomes are found exclusively in the blood vessels and not in the muscle fibers (Working et al., 1994), suggesting that most of the drug is not bioavailable in the myocardium. A comparison of cardiotoxicity in beagle dogs showed a higher incidence of cardiomyopathy and vacuolization of cardiac muscle fibers when administered as the free drug compared with TLC D-99, confirming a protective effect of liposomal encapsulation on cardiotoxicity (Kanter et al., 1993). A similar cardioprotective effect was seen with SSL DOX in both rabbits and beagle dogs (Working and Dayan, 1996; Working et al., 1999). Compared with the considerable damage observed to the myocardium of beagle dogs treated with free DOX (cumulative dose of 10 mg/kg), no histiological indication of cardiotoxicity was seen when treated with the same cumulative dose of SSL DOX (Working et al., 1999).

Clinical data are limited due to the relatively low cumulative dose of DOX delivered in most studies. In one phase I study, four patients received a total cumulative dose of >500 mg/m^2. There was no clinical cardiotoxicity or decrease in the resting left ventricular ejection fraction in any of these patients (Casper et al., 1997). In a recent study with TLC D-99 for the treatment of metastatic breast cancer (75 mg/m^2 every 3 weeks), 16% of patients on TLC D-99 had a cardiac event (either a problem with the electrocardiogram or a decrease in left ventricular ejection fraction), but there were no instances of congestive heart failure, compared with 25% of patients with cardiac events and three instances of congestive heart failure for patients receiving free DOX (Batist et al., 1998). Although significant compared with free DOX, this reduction in cardiotoxicity is not as great as expected for stable liposomes that show reduced drug leakage in the plasma. Patients with a cumulative dose of liposomal daunorubicin between 600 and 1000 mg/m^2, including one patient with a cumulative dose of >1000 mg/m^2, showed no evidence of cardiotoxicity (Gill et al., 1995). Recently, endomyocardial biopsy was used to demonstrate significantly less DOX-induced cardiac damage in patients receiving SSL DOX (cumulative doses of 440–840 mg/m^2) compared with two earlier studies with free DOX (cumulative doses of 174–671 mg/m^2; Berry et al., 1998). In another study, patients receiving 40 to 50 mg/m^2 Doxil every 3 to 4 weeks for the treatment of refractory ovarian cancer showed no decrease in the left ventricular ejection fraction (Muggia et al., 1997); this included nine patients who received >550 mg/m^2. A similar response was seen with breast cancer patients treated with Doxil (Ranson et al., 1997), with only 1 of 71 patients showing a decrease in the ejection fraction of >10%. It should be noted that the average administered dose in this final study was only 179 mg/m^2 (45–399 mg/m^2), which is well below the recommended maximum cumulative dose for free DOX. From these limited studies, it appears that Doxil, TLC D-99, and DaunoXome are significantly less cardiotoxic than the free drugs. However, the data available thus far are unable to indicate conclusively a benefit for one liposomal formulation over another. A more thorough study of the cardiotoxicity at higher cumulative doses is needed to establish a new recommended cumulative dose for liposomal formulations.

2. Vesicant Properties. The vesicant effect seen with free DOX is also markedly reduced by encapsulation in eggPC/Chol- (Balazsovits et al., 1989) or HSPC- (Gabizon et al., 1993; Oussoren et al., 1998) containing liposomes. Mice injected s.c. with free DOX showed severe necrosis, acanthosis, edema, and inflammatory infiltration when free DOX was injected, whereas only a mild edema and inflammatory infiltration were observed with SSL DOX (Gabizon et al., 1993). This suggests liposomes are able to effectively protect the skin from vesicant damage due to DOX until the carrier can be drained from the injection site by the lymph and the blood.

3. Myelosuppression. The toxicity of DOX-loaded liposomes is extremely sensitive to the rate of drug leakage from the liposome in the circulation, and thus the lipid composition (Mayer et al., 1989; Bally et al., 1990a; Oussoren et al., 1998). Liposomes composed of Chol and high-phase transition phospholipids such as DSPC or SM have rigid membranes and retain the drug well in the circulation. However, when DSPC is replaced by the more fluid eggPC, DOX is able to more readily transverse the membrane and be released into the general circulation (Hwang, 1987; Mayer et al., 1989; Gabizon et al., 1993). The free drug is generally considered responsible for most types of toxicity. Indeed, the calculated LD_{50} decreased almost 3-fold from 161 to 57 mg/kg DOX when going from DSPC/Chol (55:45) to eggPC/Chol (55:45) liposomes (Mayer et al., 1989). It should be noted, however, that the LD_{50} for eggPC/Chol (55:45) liposomes was still greater than two times that for free DOX, indicating that even encapsulation in "leaky" liposomes provides some degree of toxicity buffering. The effect of DOX on myelosuppression was also greater when encapsulated in eggPC/Chol liposomes compared with in

DSPC/Chol liposomes (Bally et al., 1990a). The decrease and recovery in the number of bone marrow cells (90% reduction on day 3) and in spleen weight were similar for free DOX and DOX-loaded eggPC/Chol liposomes (100 nm) administered at a dose of 20 mg/kg. DOX-loaded DSPC/Chol liposomes depressed the number of bone marrow cells by only 40% and were around normal levels on day 7. The effect on spleen weight was less severe, causing a 23% reduction on day 1 that returned to normal by day 14, compared with a 50% reduction for free DOX or DOX-loaded eggPC/Chol liposomes. An unusual finding was that DOX-loaded DSPC/Chol liposomes caused extended reductions in peripheral white blood cell counts (leukopenia) that were ~50% below initial values on day 14, a time at which mice treated with eggPC/Chol L-DOX had nearly returned to normal. From this study, eggPC/Chol L-DOX showed little improvement over free DOX in terms of myelosuppression. A similar study in dogs showed that free drug and TLC D-99 resulted in similar levels of myelosuppression (Kanter et al., 1993). This translated into the clinic where neutropenia and leukopenia were the most common adverse effects for both DaunoXome and TLC D-99 (Conley et al., 1993; Gill et al., 1996) and was dose limiting for TLC D-99 (Conley et al., 1993; Cowens et al., 1993; Casper et al., 1997). Myelosuppression could be partially controlled by the addition of colony-stimulating factors for bone marrow support (Casper et al., 1997).

4. Nausea, Vomiting, and Alopecia. Alopecia, mucositis, nausea, and vomiting were observed at all doses (75–105 mg/m^2) of TLC D-99 (Casper et al., 1997) but were less severe than free DOX when compared at a dose of 75 mg/m^2 (Harris et al., 1998). Although grade 3 and 4 alopecia, nausea, and vomiting were observed for DaunoXome delivered at 40 mg/m^2 every 2 weeks, it was usually <2 to 3%. Alopecia, nausea, and vomiting was rare in patients treated with Doxil at a concentration of 45 to 60 mg/m^2 (Muggia et al., 1997; Ranson et al., 1997). Myelosuppression was mild, occurring in most cycles at a grade of 2 or less (Ranson et al., 1997). Toxicity benefits were also observed in patients treated for AIDS-related Kaposi's sarcoma, where the combination of free Adriamycin, bleomycin, and VCR produced more instances and greater severity of most toxicities than Doxil (Northfelt et al., 1998).

5. Hand and Foot Syndrome (Palmar-Plantar Erythrodysesthesia Syndrome). One of the most significant toxicities for SSL DOX is a condition consisting of dermal lesions referred to as H-F syndrome) (Gordon et al., 1995; Uziely et al., 1995; Amantea et al., 1999). This same condition was previously described in patients receiving long continuous infusions of 5-fluorouracil, DOX, or vinorelbine (Lokich and Moore, 1984; Vogelzang and Ratain, 1985; Hoff et al., 1998) but is not observed in patients receiving chemotherapy by bolus injection. This particular toxicity, also called palmar-plantar erythrodysesthesia syndrome, is most likely a toxic effect of DOX on the rapidly dividing keratinocytes. Histological sections of the affected areas showed significant hyperkeratosis and parakeratosis in the stratum corneum of the epidermis (Gordon et al., 1995). Interruption of chemotherapy results in desquamation and reepithelialization of the affected areas and was complete within 3 to 7 weeks after the discontinuation of treatment (Gordon et al., 1995; Uziely et al., 1995). Interestingly, when free DOX is delivered via continuous infusion, the reversal of this syndrome was complete within 1 to 2 weeks after the discontinuation of treatment (Vogelzang and Ratain, 1985). There has been little success in reversing this toxicity without discontinuation of treatment. Discontinuation of treatment often leads to relapse of the cancer (Uziely et al., 1995). One promising strategy for the treatment of H-F syndrome without interruption of treatment involves the use of the strong reductant DHM3, which converts anthracyclines to the inactive 7-deoxyaglycone (Averbuch et al., 1985, 1986, 1988; Dorr, 1990). This agent was studied because of its capacity to reduce the vesicant effect observed with free DOX at the site of injection, but it may be useful in treating this syndrome as well. In other studies, the oral administration of pyridoxine (vitamin B_6) was shown to reduce the severity of H-F syndrome, resulting in fewer delays or discontinuations of treatment (Vukelja et al., 1989, 1993; Fabian et al., 1990; Vail et al., 1998). Topical dimethyl sulfoxide may be another method of reducing skin toxicity resulting from treatment with SSL DOX. It was previously shown to reduce vesicant damage in patients administered free DOX (Olver et al., 1988; Bertelli et al., 1995). The use of peripheral vasoconstrictors, such as ergotamine, may also potentially reduce the severity of H-F syndrome in patients treated with SSL DOX. Ergotamine is presently used in the treatment of migraine headaches (Perrin, 1995; Silberstein, 1997), but its ability to constrict blood vessels in peripheral tissues may restrict blood flow, and thus the accumulation of SSL DOX in the skin of the hands and feet. The choice and subsequent modification of the dose intensity appear to be the current anecdotal strategy for reducing the seriousness of this toxicity (Uziely et al., 1995; Ranson et al., 1997). The further study and use of compounds such as DHM3, pyridoxine, ergotamine, and dimethyl sulfoxide in combination with SSL DOX will potentially allow for dose intensification and increased antitumor efficacy.

It is possible that liposome-mediated drug delivery results in a slow-release mechanism of DOX into the tissues that mimics the effect seen with continuous infusion chemotherapy. Drug delivered by bolus injection may saturate mechanisms responsible for its uptake by the skin, where a majority of the drug can be removed from the circulation by alternative pathways: a wide tissue distribution and rapid excretion in the bile. A continuous infusion of free drug or slow release of DOX from SSL DOX results in lower peak levels of the drug in

the circulation and possibly increased uptake by the skin. Alternatively, several studies have shown that long-circulating liposomes accumulate to a limited degree in skin (Gabizon et al., 1990, 1997), where slow release in the near vicinity of keratinocytes can give rise to their toxicity. Although H-F syndrome is a serious concern, the dosage and treatment schedule can be adjusted to minimize this toxicity and still maintain a high antitumor efficacy compared with standard chemotherapy regimens (45 mg/m^2 every 3 weeks; Ranson et al., 1997).

6. Mucositis. Mucositis was also slightly increased in patients treated with SSL DOX (Gabizon et al., 1994; Uziely et al., 1995; Alberts and Garcia, 1997). Like H-F syndrome, mucositis is increased by prolonged infusion of free DOX, so its increased incidence is not surprising (Alberts and Garcia, 1997). Stomatitis was dose limiting at single doses of >70 mg/m^2 in one study (Uziely et al., 1995), but at the doses used presently (45 mg/m^2 every 3 weeks), it is mild. Although most toxicities are greatly reduced with SSL DOX, those toxicities normally associated with prolonged infusions of the free drug seem to manifest themselves in SSL DOX, most likely as a result of the long circulation lifetimes.

7. Reticuloendothelial System Impairment and Opportunistic Infections. Despite claims to the contrary (Mayer et al., 1998), several studies have shown that both SSL and CL DOX can impair the phagocytic activity of liver macrophages (Kupffer cells), as well as significantly deplete their total numbers in rats, with the use of clinically relevant doses of L-DOX (Allen et al., 1984; Daemen et al., 1995, 1997). Mayer et al. (1998) referenced a dose of liposomal DOX (2 mg/kg) relevant in some studies with SSL DOX but <10-fold the dose of CL DOX used in studies from their laboratory (Mayer et al., 1997; Parr et al., 1997). Thus, at clinically relevant concentrations of CL DOX, macrophage toxicity and depletion do appear to be serious concerns. This is not surprising because the primary route of clearance for liposomal DOX of either form is via splenic or liver macrophages (Hwang, 1987; Senior, 1987), whereas free DOX is primarily excreted in the bile (Benjamin et al., 1974; Speth et al., 1988). Thus, their preferential accumulation at these sites might be expected to result in a toxic effect. RES impairment is a serious concern, especially in immunocompromised patients, where it is the first-line defense against bacterial or fungal infections. In addition to the increased susceptibility to infection (Qian et al., 1994), macrophage toxicity has been shown to result in a decreased ability to fight metastatic growth (Levy and Wheelock, 1974; Roh et al., 1992; Heuff et al., 1993). Although in theory this is a logical concern, there are at least two studies that suggest that L-DOX may enhance the efficacy in the treatment of liver metastatic cells due to its increased localization in the liver (Gabizon et al., 1983; Mayhew et al., 1987).

Male Wag/Rij or female R-strain albino rats treated with DOX-loaded DSPC/Chol (55:45) liposomes at a dose of 5 mg/kg DOX showed a reduced ability to phagocytose unloaded liposomes (70% after a single injection or 90% after three injections) or *Klebsiella pneumoniae* bacteria (Daemen et al., 1995). A decrease in the total number of macrophages by 56 or 85% for rats treated with two or three injections of L-DOX, respectively, was also observed. Placebo liposomes (25 μmol lipid/kg) or free DOX (5 mg/kg) had no measurable effect on phagocytic activity, indicating this toxicity was carrier dependent. The dose of L-DOX used to produce a therapeutic effect with DSPC/Chol liposomes has been from 20 to 55 mg/kg DOX in rats (Mayer et al., 1997; Parr et al., 1997), or 4- to 11-fold greater than the dose used in these studies (and 10-fold greater than the dose quoted by Mayer et al., 1998). The effects at 20 to 55 mg/kg DOX would be expected to be even greater and thus significantly inhibit RES function in both liver and spleen. The saturable pharmacokinetics, shown in *II. Pharmacokinetics and Biodistribution of Liposomes and Liposomal Drug* to be responsible for increased circulation lifetimes of high concentrations of CLs, results from a partial toxicity to RES macrophages. In these studies, liposomes of identical composition and size, but lacking DOX, had more rapid clearance rates, indicating the toxicity was due to DOX (Mayer et al., 1998).

Two studies have been carried out with SSL DOX (Daemen et al., 1997; Storm et al., 1998). The first study used doses and schedules similar to those chosen for the study with CL DOX mentioned earlier (Daemen et al., 1997). Male Wag/Rij rats received a single or two or three injections, at 3-day intervals, of 5 mg/kg SSL DOX. The maximum tolerated dose for SSL DOX in humans is 60 mg/m^2 DOX at 4-week intervals for patients with Kaposi's sarcoma (Uziely et al., 1995; Coukell and Spencer, 1997), which translates to ~1.5 mg/kg DOX in rats.

It should be noted that the liposomes used in the studies with a CL formulation were ~200 nm, almost twice that of optimized formulations (Daemen et al., 1995). The lipid composition of a second study, eggPC/Chol/PEG-DSPE (55:45:5), raises questions about the bioavailability of the drug due to the source of phosphatidylcholine chosen (Daemen et al., 1997). Although these studies suggest macrophage toxicity may be a serious concern, due to the size dependence of clearance for CLs (Hwang, 1987; Senior, 1987) and the increased bioavailability of DOX from egg PC-containing SSL DOX (Gabizon et al., 1993), a study comparing the effects of optimized L-DOX formulations on splenic and liver macrophages toxicity is warranted.

Clinically, CL daunorubicin (DaunoXome) is only indicated for advanced HIV-related Kaposi's sarcoma due to the short time until observance of opportunistic infections in treated patients compared with controls (145 versus 371 days; White, 1997). In a second case, SSL DOX was responsible for one case of fatal hepatic failure

in an AIDS patient with impaired liver function (Hengge et al., 1993). Both of these cases suggest that toxicity to liver macrophages impairs bacterial or viral clearance. Although immunocompromised patients represent a special class of patients, not necessarily representative of the majority of cancer patients, the injected dose used in these studies was several fold-less than the dose needed to obtain the desired RES blockade effect required by Mayer et al. (1998). At these levels, even healthy patients may experience liver toxicity. Although this, of course, is speculative, it does suggest the need for additional toxicity studies in animals, specifically looking at the effect of L-DOX administration on RES and liver function. These studies may be more informative if formulations were optimized for size and composition of both CL and SSL DOX and at a range of relevant doses.

D. Final Comparisons of Conventional and Sterically Stabilized Liposomes

An additional problem when using CLs is the difficulty in predicting the effects of drug encapsulation on the different types of toxicity in humans based on earlier animal studies. As was mentioned previously, one of the most important advantages of steric stabilization is the dose independence provided by this particular carrier. Differences in serum opsonins between species, and thus rates of uptake by various tissues, may be more radically affected by CLs. Differences in pharmacokinetic parameters and toxicity profiles may differ not only between different animal species but also between different strains of the same animal model. Scid and immunodeficient nude mice are commonly used in antitumor efficacy studies. Scid mice have less efficient scavenging systems than normal mice and deficient DNA repair mechanisms. The high concentrations of L-DOX required to maintain long circulation times with CLs may prove especially toxic to these strains of mice and preclude their use. Even with SSLs, the dose must be scaled down significantly (1–2 mg/kg) to prevent significant drug-induced toxicities (Williams et al., 1993). This is possible with the dose-independent pharmacokinetics of SSL DOX but may not be possible with CL L-DOX.

The toxicology studies reviewed here show that liposome encapsulation offers significant protection against many common toxicities of anticancer drugs (Table 10). The degree of protection is higher when the liposomes leak their contents less readily. H-F syndrome and mucositis appear to be the most significant obstacle preventing dose escalation of the long-circulating Doxil. In animals, the dose of DOX being used in CLs (20 mg/kg) is between 3 and 20 times that being used with SSLs (1–6 mg/kg). Although some nonspecific toxicities may be slightly reduced for CL DOX (DSPC/Chol) compared with SSL DOX, DOX is unlikely to be tolerated at even three times the dose of SSL DOX in humans. The resulting increased levels of toxicity observed at the doses required to obtain long circulation will likely prevent their use in multidose regimens at these concentrations. It is unknown whether the drug-induced RES blockade required to obtain long circulation times will be maintained at schedules requiring lower doses and multiple injections.

In any event, independent of the liposome formulation, entrapment of DOX inside liposomes significantly alters the toxicity profile of DOX. This altered profile makes the liposomal drugs more tolerable, preventing patients from leaving treatments due to unbearable toxicities. Another related issue is the increased quality of life. Although alopecia, nausea, and vomiting are severe with many standard chemotherapeutic agents, they are rare or significantly reduced among patients treated with L-DOX. The toxicity buffering provided by liposomes is a considerable improvement in itself over standard chemotherapy.

VII. Stability in Plasma and Storage

The stability of drug-loaded liposomes over time is an important concern in pharmaceutical formulations. Stability can refer to several different aspects of a liposomal drug formulation: chemical stability of both drug and lipid components, colloidal stability, and drug retention. For applications of liposomes where specific delivery of liposome-associated drug to solid tumors is desired, liposomes must substantially retain their contents while in the circulation (Senior, 1987). In other applications, such as the delivery of photosensitizers to tumors in photodynamic therapy, liposome-associated photosensitizers immediately redistribute to other hydrophobic sites, such as plasma lipoproteins in the circulation, which in turn accumulate in tumors (Allison et al., 1990; Reddi, 1997). Various factors can affect the relative stabilities of such preparations in the presence of plasma. This plasma-induced destabilization is exquisitely sensitive to the lipid composition of the liposome. To be more attractive for pharmaceutical development, liposomal drug formulations also must be stable during prolonged storage. Liposomes have either been stored preloaded with DOX, as is the case for PEG-coated liposomes, or as "empty" liposomes that are loaded by a pH gradient immediately before injection (Madden et al., 1990; Haran et al., 1993; Lasic et al., 1995; Cullis et al., 1997). Compositions containing more fluid lipid components, such as eggPC, require remote-loading just before injection, due to a high level of leakage during storage.

A. Physical Stability of Liposomal Drug Formulations

For amphipathic drugs that can readily cross membranes, there are a variety of factors that can influence the stability of a liposomal formulation. The presence of Chol and saturated phospholipids appear to be the most important factors for reducing membrane permeability of these drugs (Bally et al., 1990b; Gabizon et al., 1993). Other factors, such as the drug-loading method, which

can result in internal concentrations of the drug exceeding the aqueous solubility of the free drug, also act to stabilize the formulation.

Cholesterol appears to be especially important in stabilization of liposomes to the effects of plasma components such as HDL (Mayhew et al., 1979; Allen and Cleland, 1980). In addition, several early studies have indicated that Chol was essential for controlling the permeability properties of membranes to ions or small molecules (Papahadjopoulos et al., 1972, 1973a,b). The presence of Chol in a 1:1 ratio with PG and PC was shown to reduce the amount of ara-C leakage observed in the presence of serum from 88 to 28% after 24 h in one study (Mayhew et al., 1979). HDL has been shown to destabilize pure PC liposomes by catalyzing the net exchange of PC from liposomes to HDL particles (Scherphof et al., 1978; Chobanian et al., 1979; Damen et al., 1980). The addition of Chol to liposome formulations results in an increase in plasma stability, inhibiting transfer of lipid components to plasma lipoproteins (Allen, 1981; Damen et al., 1981).

In vitro stability studies using human plasma or serum have some inherent limitations. Plasma is often isolated in the presence of calcium chelators to prevent blood coagulation and results in some uncertainty because calcium can often modulate interactions of proteins with membrane surfaces and, with some formulations, interact with membranes directly, causing destabilization. Although plasma can be isolated in the presence of heparin, heparin may also affect protein interactions with membranes. In addition, there is considerable interpatient variability in the levels of plasma proteins and lipoproteins, adding another level of complexity to these in vitro studies. The most relevant studies of liposome stability are completed in vivo, simultaneously monitoring the concentrations of both the encapsulated drug and a nonexchangeable lipid marker. With chemotherapeutic drugs, such as DOX, that are removed rapidly from the circulation, the drug/lipid ratio becomes an excellent measure of the stability of the formulation. Measurement of free and L-DOX after cation exchange or size-exclusion chromatography is not reflective of liposome stability because the free drug is rapidly removed from the circulation, resulting in an underestimation of the amount of drug leakage.

The in vivo leakage of DOX from DSPC/Chol (55:45) and eggPC/Chol (55:45) was measured in mice by following the clearance of a lipid label [^{3}H]cholesterylhexadecyl ether and DOX from the circulation (Bally et al., 1990b; Mayer et al., 1998). Although the DSPC/Chol formulation proved relatively stable, releasing <10% of the encapsulated DOX in 24 h, the eggPC/Chol formulation released almost 50% of its DOX within 1 h and ~70% by 4 h. A PEG-DSPE/HSPC/Chol DOX formulation appears to have even greater stability with little apparent leakage in the first 24 h and <10% leakage up to 72 h after injection (Gabizon et al., 1993). It should be noted that in the first study, DOX was loaded into liposomes by the pH gradient method of Mayer, Cullis, and coworkers, whereas in the second study, DOX was loaded according to the ammonium sulfate method. These loading methods are discussed in more detail in *VIIA1. Drug-Loading Methods*. However, differences in the loading methods, including a more rapid dissipation of the pH gradient, in the case of first method, and the formation of a stable drug-sulfate gel in the liposome interior of liposomes loaded using the ammonium sulfate method (Lasic et al., 1992a, 1995), may result in greater stability for liposomes loaded with DOX via the second method (Frézard, 1994; Frézard et al., 1994). Because the kinetics of tumor accumulation are more rapid than the rate of DOX release from liposomes loaded via either method, it is not known whether a further increase in stability is desirable or may simply act to limit the bioavailability of the drug in the tumor. Also, for amphipathic compounds such as DOX the choice of a saturated phospholipid component, such as DSPC or HSPC, is essential in maintaining a stable formulation in the circulation. The substitution of SM for phosphatidylcholine may also increase the liposome stability of some drug formulations (Parr et al., 1994; Webb et al., 1995). Intermolecular hydrogen bonding between the Chol hydroxyl group and the neighboring amide nitrogen of SM gives rise to a tightly packed bilayer that likely resists drug permeation (Schmidt et al., 1977; Smaby et al., 1996).

1. Drug-Loading Methods. A diagram depicting the ammonium sulfate remote-loading procedure is given in Fig. 9 (Haran et al., 1993; Lasic et al., 1995). Lipids are typically hydrated to form suspensions in high concentrations of ammonium sulfate (250 mM) and subsequently extruded to the desired size. Unencapsulated ammonium sulfate is removed (for example using a size-exclusion column), and the drug is added to the liposomes. Although ammonia can freely pass through membranes in its neutral form, sulfate is trapped in the liposomal lumen. When ammonia moves out of the liposome going with the concentration gradient, a hydrogen ion is left behind and a self-sustaining pH gradient is formed; DOX moves in its neutral form in the opposite direction and becomes protonated, eventually forming an insoluble salt with the entrapped sulfate anions. The resulting gel helps stabilize the drug in the interior. The cooling that occurs after the loading step, which is performed at 55–60°C, also likely plays a role in solidifying the drug precipitate, and thus increasing the stability of the formulation. A pH gradient strategy for loading weak bases was reported initially by Nichols and Deamer (1976) and later used extensively by Cullis and coworkers, with a pH gradient to drive the accumulation of drugs into liposomes (Mayer et al., 1985; Madden et al., 1990; Harrigan et al., 1993; Cullis et al., 1997). Weak acids can be loaded in an analogous manner using calcium acetate or reverse pH gradients (Clerc and Baren-

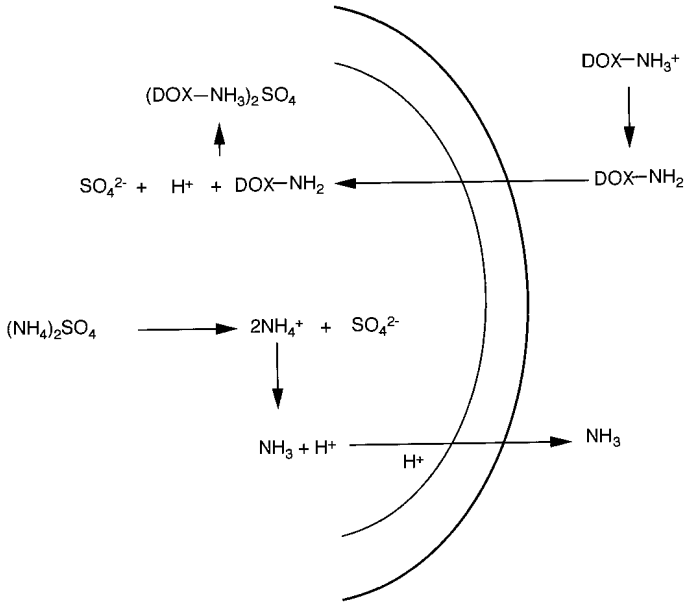


FIG. 9. Ammonium sulfate-loading procedure for weak bases. Liposomes are first prepared in the presence of ammonium sulfate (250 mM). On removal of the exterior ammonium sulfate on a size-exclusion column, DOX is added to the extraliposomal media. Ammonium sulfate can dissociate to two ammonium cations and one sulfate anion. Ammonia (NH_3) is free to cross the liposomal membrane, giving rise to a pH gradient across the membrane. DOX in its uncharged form can then cross the liposome membrane and form an insoluble gel under acidic conditions with the remaining sulfate anion on cooling, effectively trapping it in the liposomal interior. The concentration of DOX in the liposomal lumen can reach concentrations in excess of the aqueous solubility of DOX. This loading procedure can be applied to a variety of weak bases, such as those composing the anthracyclines, *Vinca* alkaloidsm or camphothecins. However, the stability of the complex formed with sulfate and thus the gel in the liposomal lumen may help determine the overall stability of the formulation.

holz, 1995; Cullis et al., 1997). These gradients also help stabilize formulations and reduce leakage during storage and while in the circulation. The small difference between DSPC/Chol (55:45) and HSPC/Chol/PEG-DSPE (92.5:70:7.5) in the observed amount of in vivo DOX leakage is possibly due to differences in the remote loading procedure (Gabizon et al., 1993; Mayer et al., 1998). Most studies with SSLs typically use ammonium sulfate gradients to entrap amphiphatic basic amines such as DOX, whereas studies using CLs prefer the pH gradient method (Madden et al., 1990; Haran et al., 1993; Lasic et al., 1995; Cullis et al., 1997).

2. Physical Stability of Liposome Formulations with Nonanthracyclines. An excellent review recently described the relationship of drug structure and physical properties of the liposomal membrane to drug-loading efficiencies and the stability of liposome-drug formulations (Barenholz, 1998). The drugs described thus far are considered membrane active, meaning they are amphipathic in nature and able to insert into and transverse both artificial and biological membranes. Other drugs, such as *N*-(phosphonoacetyl)-L-aspartate, have a more polar character and are unable to freely transverse membranes (Heath and Brown, 1989). Stable liposome formulations with such drugs can include monounsaturated or polyunsaturated phospholipid components that were previously considered undesirable with drugs such as DOX or VCR. However, although it becomes easier to prepare stable formulations with highly water-soluble drugs, it also becomes more difficult to release the drug from the carrier at the tumor site where it can elicit its desired response. This aspect in investigated in more detail in *VIII. Accumulation of Liposomal Drugs in Tumors*. An additional concern with highly water-soluble drugs is how to entrap them at very high efficiencies in liposomal carriers. Methods for entrapping amphipathic drugs using a pH gradient or ammonium sulfate gradient are dependent on the partition coefficient of the drug between the aqueous phase and the liposomal membrane (Lasic et al., 1995; Cullis et al., 1997). Amphipathic drugs that partition to a greater extent in the liposomal membrane are more readily entrapped in the liposomal interior. Highly water-soluble drugs are likely to be loaded only by passive encapsulation, which is limited by the entrapped volume, and with 100-nm vesicles rarely exceeds 95% of the total volume (Szoka and Papahadjopoulos, 1978; Mayer et al., 1985).

It should be emphasized that results obtained with DOX cannot necessarily be extrapolated to other drugs, even those similar in chemical structure. For example, VCR-loaded liposomes loaded by the pH gradient method are significantly less stable in the circulation, losing ~85% of the encapsulated drug in a 24-h period (Mayer et al., 1998). Apparent rates of elimination of VCR and ara-C are also affected by membrane stability (Mayhew et al., 1979; Webb et al., 1995). When SM and Chol are included in these formulations to reduce leakage of the drug, the drug shows higher plasma levels and reduced rates of elimination from the circulation.

The presence of PEG-DSPE may decrease the stability of some liposomal drug formulations (Webb et al., 1995, 1998). SM/Chol liposomes containing entrapped VCR were shown to rapidly leak VCR in the circulation in the presence of PEG-DSPE. We have observed reduced loading of vinorelbine, another *Vinca* alkaloid, at high mol% value of PEG-DSPE (5–6 mol%; Kirpotin et al., 1999a). However, reducing the concentration of PEG-DSPE to 3 mol% significantly increased the loading efficiency. Thus, the stability dilemma observed with some drugs in the presence of PEG-DSPE may be overcome by simply reducing the concentration of PEG-DSPE, but the effect of reducing the concentration of PEG-DSPE has on the pharmacokinetics of the liposome remains to be seen. A simple increase in the molecular weight of PEG used from 2000 to 5000 may result in a similar degree of protection to that observed with the lower molecular weight PEG at a higher mol% value. Alternatively, Mayer and coworkers suggested the stability problem is a consequence of the negative charge found at the membrane interface with PEG-DSPE (Webb et al., 1998). The exchange of PEG-DSPE for a neutral PEG-ceramide con-

jugate resulted in greater stability of liposomal VCR preparation.

3. Drug/Lipid Ratio. An optimal drug/lipid ratio is known to be important in the development of a stable formulation. The drug/lipid ratio should be as high as possible to maximize the payload of drug reaching the tumor without compromising stability. The maximum amount of drug loaded per liposome is dependent on the method used for drug loading, the size of the liposome, and the presence of trapping components such as acidic lipids to which the drug can bind. Because the latter two factors are traditionally associated with negative effects on pharmacokinetic parameters, the drug-loading method is the most readily adjustable. For passive encapsulation of daunorubicin in CLs, the concentration achieved was 0.079 μg drug/μg lipid. For remote loading via a simple pH gradient, the most effective concentration reached was 0.250 μg drug/μg lipid, and for remote-loading using an ammonium sulfate gradient, it was 0.125 μg drug/μg lipid (Table 1). Drug/lipid ratios that are too high can also form less stable formulations, presumably due to the dissipation of the pH gradient during drug loading (Mayer et al., 1990c, 1993). These results emphasize the care needed in optimization of drug-loading methods to prepare stable liposomes and at the same time maximize encapsulation efficiencies.

4. Osmolarity Effects. Several studies have investigated the role of osmolarity on the development of stable liposomal drug formulations (Allen et al., 1992, 1995b; Mui et al., 1993, 1994). Allen et al. (1992) showed that entrapped ara-C was released more rapidly when entrapped under hyperosmotic conditions, and its release was characterized by initially rapid kinetics, followed by a slower second rate of leakage. This is consistent with the work of Madden and coworkers, who showed that osmotic lysis results in only partial release of liposomal contents and that after resealing of the liposome membrane, the liposomal lumen remains hyperosmotic (Mui et al., 1993, 1994). VCR-loaded SSLs loaded using an ammonium citrate gradient were relatively stable (leakage $T_{1/2}$ = 84 h) when loaded under iso-osmotic conditions (125 mM ammonium citrate; Allen et al., 1995). In contrast, 90% of the encapsulated VCR was released after 24 h from DSPC/Chol liposomes loaded via the pH gradient method under hyperosmotic conditions (400 mM sodium citrate; Boman et al., 1994). A fine balance may exist between the osmotic stability of the liposome, residual pH gradients after loading, and the formation of drug precipitates in the liposomal lumen. For the pH gradient drug-loading method, a high buffer capacity is typically required in the intravesicular medium to maintain a reasonable pH gradient and obtain high amounts of drug loading (Mayer et al., 1990c; Boman et al., 1993; Cullis et al., 1997). High concentrations of DOX form gel-like precipitates with low osmotic activity (Lasic et al., 1992a; Haran et al., 1993). For example, DOX loaded into PEG-DSPE/DSPC/Chol or DSPC/Chol liposomes, using either the pH (400 mM sodium citrate) or ammonium sulfate (250 mM ammonium sulfate) gradient method, is stably encapsulated in the presence of plasma. However, other drugs that form less stable complexes or gels may still have a considerable osmotic gradient, after the drug-loading process, that can increase further during drug loading (Boman et al., 1993). For instance, both daunorubicin and VCR have a considerably greater aqueous solubility than DOX (Madden et al., 1990), and both leak at a faster rate than DOX (Boman et al., 1993; Haran et al., 1993; Mayer et al., 1993). Other factors, such as the pK_a of titratable groups on the drug, a more rapid dissipation of the pH gradient, and the ability of the soluble form of the drug to partition more readily into the liposome membrane, as opposed to drug precipitates or crystals, also likely play a role in the decreased stability of such formulations relative to DOX (Madden et al., 1990; Mayer et al., 1993; Cullis et al., 1997).

5. Stabilizing against Aggregation. Although more solid CLs composed of DSPC and Chol leak drug very slowly, they are difficult to work with due to increased flocculation and aggregation over time (Crommelin, 1984; Gamon et al., 1989; Barenholz et al., 1993). Early preparations were often stabilized with small quantities of negatively charged lipids such as PG to prevent aggregation from occurring during storage (Gabizon et al., 1983, 1986, 1989). However, as was previously discussed, the presence of certain anionic phospholipids increases the rate of clearance from the circulation (Hwang, 1987; Senior, 1987). The presence of PEG on the surface provides a steric barrier that prevents liposome aggregation. PEG-coated liposomes are stable with respect to both size and drug-encapsulation over the period of many months to years when stored below the phase transition of the PC component (Haran et al., 1993; Lasic and Needham, 1995).

B. Chemical Stability of Drugs and Lipid Components

Thus far, we have been primarily concerned with the physical stability of liposomal drug formulations, either in storage or in the circulation. However, another important concern is the chemical stability of both the drug and lipid components (Barenholz et al., 1993). Are the drugs and lipid components compatible with the remote loading techniques used? If ligand-mediated targeting results in endocytosis of the liposome, is the drug stable in the low pH environment of late endosomes and lysosomes or in the presence of degradative enzymes present in these structures? These are important questions that must be answered when designing a liposomal drug delivery system. When ara-C-loaded liposomes were targeted to cells in vitro, the uptake and delivery to the lysosome resulted in degradation of the drug (Huang et al., 1993). In contrast, DOX is relatively stable and able to escape the harsh conditions of the lysosome intact (Barenholz et al., 1993).

Many drugs and lipids are susceptible to base hydrolysis. A calcium-acetate gradient has been used to load amphipathic weak acids into liposomes (Clerc and Barenholz, 1995). This method presumably generates a very high internal pH. When using this method, the stability of the drug must be considered. DOX, paclitaxel, topotecan, and other drugs can be hydrolyzed under basic conditions (Ringel and Horwitz, 1987; Barenholz et al., 1993; Burke et al., 1993; Chabner and Longo, 1996). The lactone ring of topotecan is readily hydrolyzed at even neutral pH, giving rise to serious stability concerns under basic conditions (Burke et al., 1993; Subramanian and Muller, 1995), although entrapment in liposomes with an acidic interior has been shown to stabilize topotecan formulations (Burke and Gao, 1994). Finally, the fatty acid esters are sensitive to both acid and base hydrolysis giving rise to membrane-destabilizing lysolipids under certain conditions (Barenholz et al., 1993; Zuidam et al., 1995). It is wise to analyze the lipid components of a newly developed liposome formulation by thin-layer chromatography or HPLC to be confident in the chemical stability of the lipids used.

Lipid peroxidation is another important concern for unsaturated lipid components. Lipid peroxidation can be initiated by a variety of different factors and can lead to the formation of membrane-destabilizing secondary oxidation products such as 4-hydroxynonenal and malondialdehyde (Frankel, 1987a,b; Barenholz et al., 1993). Phospholipids containing diunsaturated fatty acyl chains such as linoleic, linolenic, or arachidonic acid are particularly susceptible to lipid peroxidation due to the ready abstraction of hydrogen radicals from doubly allylic carbons (Frankel, 1980, 1985). Linolenate- and arachidonate-containing phospholipids are the most likely to form complex secondary oxidation products that are particularly damaging to membranes (Frankel, 1987a,b). This brings up an important point concerning the use of unsaturated lipids. There may be liposomal drug delivery scenarios in which a more fluid membrane is preferred. When the use of unsaturated lipids is required, it is the gel-to-liquid crystalline phase transition (T_m) that is often an excellent predictor of bilayer fluidity. Table 2 gives the primary phase transitions for several different phosphatidylcholines. Increasing the acyl chain length gives rise to a higher T_m whereas increasing the number of unsaturations decreases the T_m. Thus, a lipid component with the desired T_m can be found by balancing the acyl chain length and the number of unsaturations found in a particular phospholipid component. eggPC is a widely used fluid phase lipid component that is in the liquid crystalline state at physiological temperatures. Unfortunately, it also contains a high proportion of fatty acyl groups with multiple unsaturations (18% with two olefins and 3% with four olefins), making it particularly susceptible to oxidation. As can be seen from Table 2, POPC has a comparable T_m value with only one olefin in one of the two acyl chains. eggPC was originally used because it was readily available and relatively inexpensive. It is now being used for mostly historic reasons, because most investigators prefer to continue using what is familiar to them in the literature. However, improvements in organic synthetic methods for phospholipids have led to the increased availability of synthetic lipids such as POPC and resulted in a cost that is comparable to the natural product. Combined with an increased chemical stability, POPC becomes a far more appropriate candidate for use as the unsaturated lipid component of a liposome formulation than eggPC.

The stability of a liposomal formulation is dependent on many physical and chemical factors, ranging from the individual drug and lipid components to the stable encapsulation of the drug within the carrier. A rigorous undertaking is necessary in developing any new liposomal drug formulation to ensure these stability considerations are addressed. In *VIII. Bioavailability of Encapsulated Drug*, we discuss how to balance stability in the circulation with release from the carrier on reaching the tumor.

VIII. Bioavailability of Encapsulated Drug

It is important to emphasize that most of the work described thus far has been concerned with drugs considered to be membrane active. They are amphipathic in nature and able to transverse the bilayer at a rate dependent on the physical properties of the membrane, as well as any ionic or pH gradients across the membrane (Madden et al., 1990; Lasic et al., 1995; Cullis et al., 1997). Other drugs, such as ara-C, are more water soluble and after a slow release from the carrier (Allen et al., 1992) can be taken up by specific transporters located in the plasma membrane of tumor cells, such as the nucleoside transporter (Plageman et al., 1978; Wiley et al., 1982) or the reduced folate carrier (Westerhof et al., 1991, 1995; Antony, 1992). The bioavailability of such compounds is dependent on how readily they are able to escape their liposomal carrier. We define bioavailability in the case of liposomal carriers as the amount of free drug that is able to escape the confines of the carrier and is thus available for redistribution to neighboring tissues and tumor. A fine balance is required to prevent premature leakage in the circulation, and thus nonspecific toxicities, but still allow for release of the drug on reaching the tumor. For DOX-loaded slow-release liposomes (PEG-DSPE/HSPC/Chol or DSPC/Chol), the drug is thought to leak very slowly and thus be similar to a slow infusion of the drug specifically near the cancerous cells (Horowitz et al., 1992; Vaage et al., 1998). Using scanning confocal fluorescence microscopy to look at s.c. implants of a prostate carcinoma xenograft, DOX delivered via SSL DOX was shown to reside immediately adjacent to tumor capillaries and venules at early times (1 h; Vaage et al., 1998). At 24 h,

DOX had leaked from the liposome and was found within the tumor in a pattern indicating diffusion away from the capillaries and venules. Free DOX was found deep within the tumor at 1 h but was nearly undetectable at 24 h. This is likely due to both elimination and metabolism of the drug, as well as fluorescence quenching after intercalation of the drug into nucleic acids (Gigli et al., 1988). These results indicate that DOX does become bioavailable on reaching the tumor, where it slowly and continuously bombards the nearby cancer cells with low levels of the cytotoxic agent. Thus far, most detailed studies have used anthracyclines for delivery studies. Although the antitumor cytotoxicity of drugs such as anthracyclines and ara-C are less dependent on peak levels of the drug, cytotoxicity of other drugs may show a considerably greater dependence on peak levels of the drug, and hence the rate at which the drug is released from its carrier. Consequently, the selection of drugs with these properties or the selective increase in the rate of release at the tumor site will be very important in designing an effective carrier.

A. Release of Doxorubicin in Tumor

The mechanisms responsible for liposome breakdown and drug release in tumors have not been well elucidated. Several potential mechanisms have been proposed, but all are highly speculative and little direct evidence has been provided, primarily due to technical difficulties associated with monitoring drug release in vivo. Some of the properties of the tumor microenvironment believed to play a role in liposome destabilization include the slightly acidic pH found in interstitial fluids surrounding tumors, lipases released from dying tumor cells, inflammatory cells present in response to tumor release factors, enzymes, and oxidizing agents (Martin, 1998). In addition, phagocytic cells residing in tumors could metabolize liposomes and release free DOX, killing neighboring tumor cells via the bystander effect (Storm et al., 1988). The effect of local interstitial media on DOX leakage from SSL DOX was investigated in an in vitro study (Gabizon, 1995). Although leakage in plasma was relatively slow ($T_{1/2} \sim 100$ h), liposomes incubated in the presence of fluid obtained from pleural malignant effusions leaked DOX at a significantly elevated rate. Another investigator suggested that ammonium sulfate used to remote loaded DOX could also catalyze liposome breakdown, although a logical rationale for its mechanism was not provided (Lasic, 1993). With SSLs, a certain amount of PEG-DSPE can be released from the liposome over time, allowing liposomes to undergo more interactions with neighboring cells and or plasma components. Finally, it may be possible that DOX passively crosses the liposome membrane and that as the DOX-sulfate gel is gradually destabilized by loss of more and more drug, the drug release is accelerated. Finding methods to selectively destabilize liposomal drug formulations in the tumor area is a major challenge to the liposome field, which if overcome could lead to substantial increases in drug bioavailability at the tumor site and subsequent increased efficacy.

The release of DOX from eggPC/Chol liposomes is rapid compared with liposomes composed of HSPC/Chol or DSPC/Chol (Bally et al., 1990b; Gabizon et al., 1993). eggPC/Chol liposomes release a significant portion of their drug before reaching the tumor and thus act as a rapid-release system (Harasym et al., 1997), in contrast to the more stable formulations that act as slow-release systems and are the focus of this review.

B. Active Targeting of Liposomes

Although clearly more beneficial than the use of free DOX, one disadvantage of SSL DOX or L-DOX is that cancer cells deep within the tumor are not readily reached with high concentrations of drug and are given an opportunity to select for drug-resistant cells. One strategy for increasing drug bioavailability and distribution within the tumor has been to target liposomes to internalizing receptors. Liposomes have been targeted to cells via small molecules (Lee and Low, 1994, 1995), sugar molecules (Spanjer and Scherphof, 1983; Banerjee et al., 1996), serum proteins (Afzelius et al., 1989; Brown and Silvius, 1990; Lundberg et al., 1993), and antibodies (Heath et al., 1983; Debs et al., 1987; Matthay et al., 1989; Maruyama et al., 1990a; Allen et al., 1995c; Lopes de Menezes et al., 1998) or antibody fragments (Park et al., 1995; Kirpotin et al., 1997b). Recently, HER2-targeted immunoliposomes were shown to distribute within solid tumors and not simply in the extracellular space surrounding the tumor blood vessels (Kirpotin et al., 1997a, 1999a; Park et al., 1997). Release of the drug within the tumor itself presumably increases the bioavailability of the drug to the more-difficult-to-reach cells within the solid tumor mass. Indeed, this property is most likely responsible for the increased therapeutic effect observed with these carriers, as there was no overall increase in liposome localization to the tumor (Fig. 10).

Active targeting of pharmaceuticals is often perceived as a means of getting increased amounts of drug into the diseased site. However, the passive trapping of liposomes due to a discontinuous tumor microvasculature, the lack of a functioning lymphatics, and a high interstitial pressure result in a rate-limiting accumulation of liposomal drug in solid tumors. It is unlikely that active targeting to cell surface proteins of solid tumors that are not internalized will offer a significant therapeutic benefit. When anti-HER2-targeted immunoliposomes are prepared with an antibody that is not internalized, there was no increase in therapeutic efficacy compared with nontargeted liposomes (Goren et al., 1996). Similar to the results seen with the internalizing anti-HER2 Fab' fragment (Kirpotin et al., 1998; Park et al., 1998a,b), there was no increase in tumor levels of the targeted liposomes compared with nontargeted liposomes (Goren

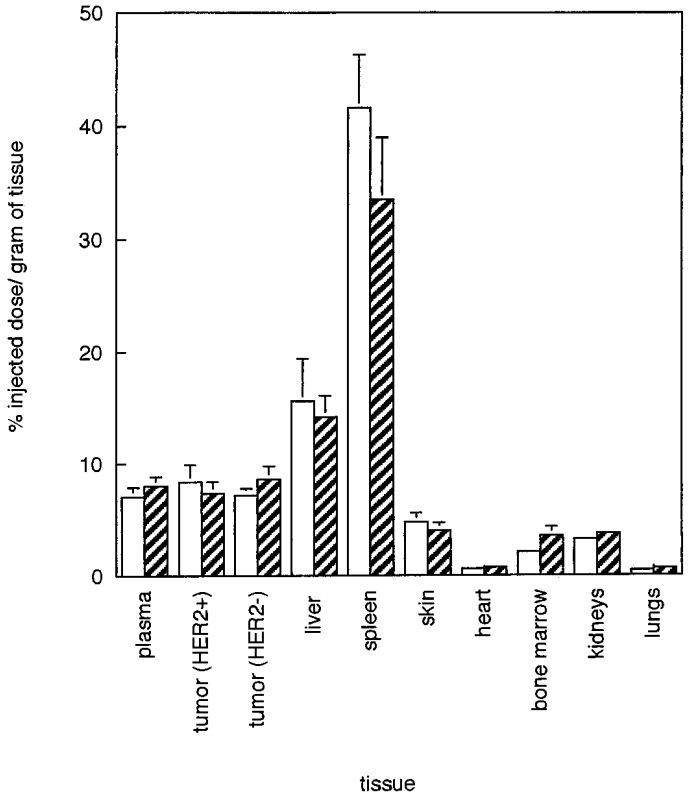


FIG. 10. Tissue distribution of anti-HER2 targeted SSLs (□) and nontargeted SSLs (rhbox) in nude mice with breast cancer xenografts. Xenografts were derived from both HER2-overexpressing (BT-474) and HER2-negative (MCF-7) cell lines. The biodistribution was determined at 24 h by following ^{67}Ga-labeled liposomes after injection of 1 μmol of phospholipid via the tail vein in nude mice. This figure was adapted from Kirpotin et al. (1998).

et al., 1996). Vingerhoeds et al. (1996) also failed to show increased efficacy of noninternalizing immunoliposomes targeted against the OA3 antigen present on 90% of human ovarian carcinomas. Some investigators have even suggested that cell surface binding by itself may serve to limit the distribution of liposomes within the tumor (Weinstein et al., 1987; Jain, 1989). Allen et al. (1995c) showed that SSL DOX was more effective than sterically stabilized immunoliposomal DOX targeted against a carbohydrate epitope on an ovarian cancer cell line grown s.c. in nude mice. The authors suggested the reduced activity may be due in part to the binding-site barrier. However, the circulation $T_{1/2}$ values of the immunoliposomes in this study were significantly shorter than those for the nontargeted SSL, and there was no evidence presented showing that these liposomes were internalized, giving rise to two alternative explanations for the reduced activity. Furthermore, this study used whole antibodies for targeting. In our studies with the anti-HER2 antibody, antibody fragments were used: either Fab′ or single chain FV fragments (Fig. 11; Park et al., 1995, 1998a,b; Kirpotin et al., 1998; Papahadjopoulos et al., 1999). In addition to the advantages associated with reduced immunogenicity of antibody fragments, the reduced avidity of the fragments for their cell surface targets may serve to reduce the binding-site barrier, allowing a deeper penetration of the carrier within the tumor. A deeper penetration of antibody fragments compared with full antibodies has been previously attributed to both the reduced size of the molecule and a reduced avidity for its target (Fujimori et al., 1989; Yokota et al., 1992). This, of course, is speculation, and additional studies must be completed to determine more precisely the mechanisms responsible for regulation of the tumor penetration of targeted liposomes.

Allen and coworkers have also been successful in targeting liposomes to a lung metastatic cancer model, where cancer cells travel through the blood and localize in the lung as small tumor colonies (0.5 mm; Ahmad et al., 1993; Allen et al., 1995c). An increased localization to tumor-bearing lungs was seen with targeted immunoliposomes compared with nontargeted SSL, and this correlated with a significant decrease in the tumor burden of mice treated with immunoliposomes (Ahmad et al., 1993). Cancer cells in this metastatic model differ greatly compared with the solid tumors described earlier due to their small size and the greater accessibility of liposomes to their receptor. This same group has also been successful in targeting liposomes against hematological cancers, such as B cell malignancies (Allen et al., 1995c; Lopes de Menezes et al., 1998), where the tumor cells are also more available for binding to targeted liposomes. Huang and coworkers have targeted the pulmonary endothelium using antibodies directed against the lung endothelial protein thrombomodulin (Maruyama et al., 1990a,b; Mori et al., 1993, 1995). This type of organ-specific targeting allows liposome-associated drug to be delivered near the site of tumors located in the lung, where on their disassociation from the carrier they can act on neighboring tumor cells (Mori et al., 1995). The greater accessibility of the receptors in each of these approaches offers a significant advantage for targeted therapies compared with the treatment of solid tumors.

The choice of targeting ligand is important when designing targeted liposomes. The ligand should be relatively specific for cancer cells, especially in contrast to cells readily accessible in the general circulation, where many passes may occur before extravasation into tumors. Second, as mentioned, the epitope bound should result in internalization of the liposome. Binding to a receptor that is known to be endocytosed does not necessitate endocytosis, especially in the case of antibodies or antibody fragments (Goren et al., 1996). Ligands, such as folate, for internalized receptors usually induce endocytosis, but binding of a protein or peptide to an unrelated part of the receptor may simply constrain the carrier on the membrane surface. An additional problem with the attachment of targeting molecules to the surface of liposomes is that they may increase liposome clearance by tissues other than the tumor. For instance, early studies indicated antibody-targeted liposomes are

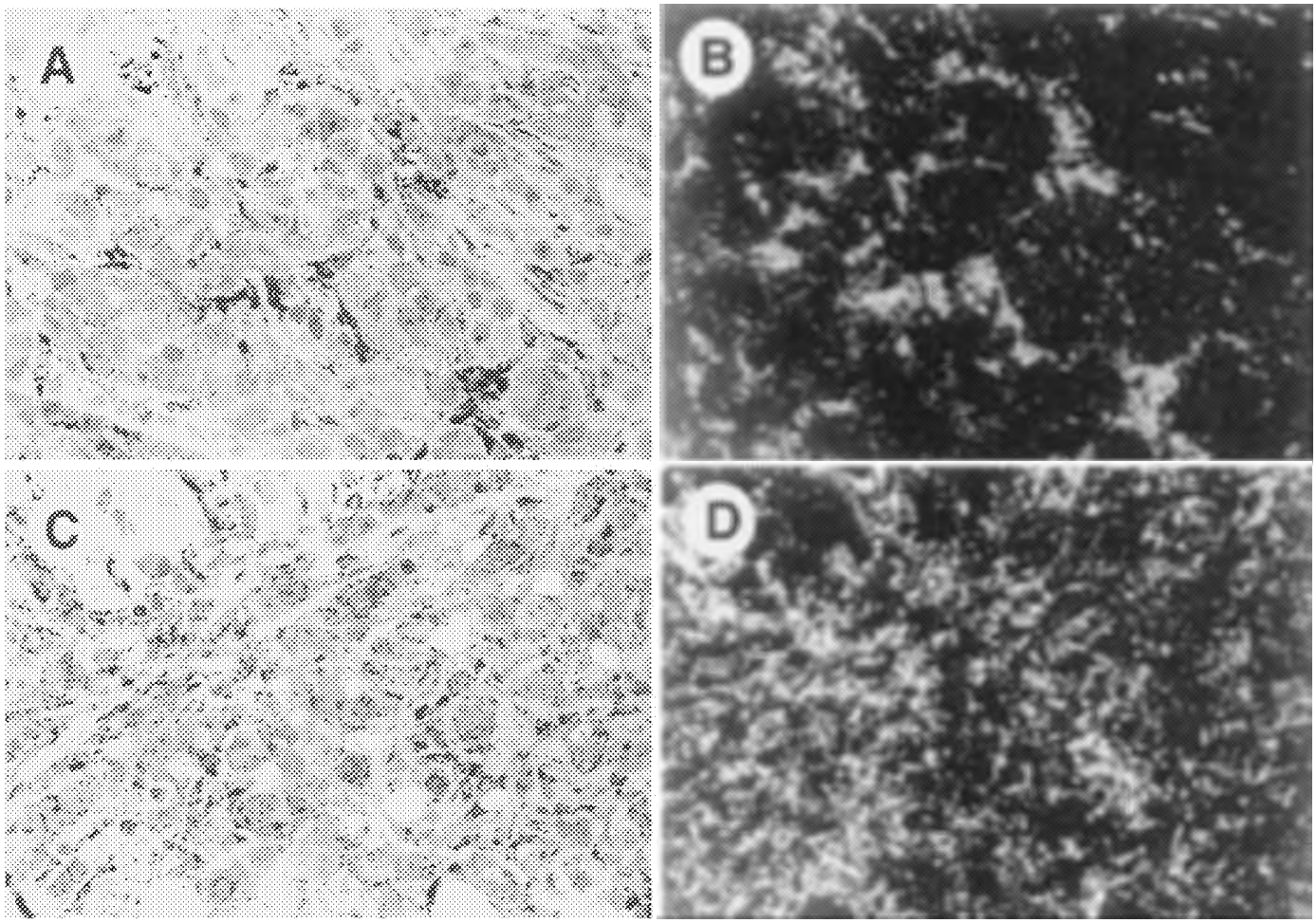


FIG. 11. Effect of tumor cell targeting on the localization of SSLs in HER2-overexpressing human breast cancer xenografts (BT-474). A and B, nontargeted SSLs. C and D, anti-HER2 SSL. Liposomes labeled with entrapped colloidal gold were injected i.v. into the mice with s.c. xenograft tumors (200–300 mm^3 in size) at the dose of 5 mmol of phospholipid/animal. Twenty-four hours later, the tumors were harvested, and liposomes were visualized on tumor sections by the silver enhancement method. Liposomes appear as bright dots on the dark field images (B and D) or as black grains on the matching bright-field images counterstained with light H&E (A and C). Nontargeted liposomes are concentrated in the interstitial spaces within the cells with characteristic macrophage morphology. HER2-targeted SSL are more uniformly dispersed throughout the tumor tissue and often found within tumor cells.

rapidly removed from the circulation by macrophages of the RES (Debs et al., 1987). This was likely due to recognition of the Fc portion of the antibody by Fc receptors located on the surface of macrophages (Aragnol and Leserman, 1986; Raghavan and Bjorkman, 1996) or by recognition of noncompatible portions of the antibody by antibodies of the humoral immune system. The recent endeavors with Fab′ targeted liposomes do not contain the Fc region of the antibody and are prepared from humanized versions of the antibody (Park et al., 1995; Kirpotin et al., 1997b). Indeed, these immunoliposomes have a nearly identical tissue distribution as that of nontargeted SSL DOX (Fig. 10). A more in-depth description of these immunoliposomes and the various properties necessary for their optimization is given in several recent reviews (Kirpotin et al., 1997a, 1998; Park et al., 1997). There also are many reviews describing the methods for preparing and applying other targeted liposomes (Allen and Moase, 1996; Allen et al., 1997, 1998; Forssen and Willis, 1998; Park et al., 1998a,b). The most relevant aspect of targeted liposomes is that targeting to internalizing receptors can potentially increase the bioavailability of the drug. It can accomplish this by altering the intratumoral distribution of the liposome and thus increasing the percentage of cells exposed to the drug. This effect has only been observed for HER2-targeted immunoliposomes and may very well differ depending on the targeting ligand.

Liposomes targeted to internalizing receptors have shown considerably greater tumor cell cytotoxicity both in vitro and in vivo (Heath et al., 1983; Huang et al., 1983; Berinstein et al., 1987; Matthay et al., 1989; Lee and Low, 1995; Park et al., 1995, 1997; Lopes de Menezes et al., 1998). This may be due in part to an increased bioavailability after transport of the liposomes to lysosomes, where degradative enzymes can breakdown the liposomal membrane and release the drug. Many studies have demonstrated degradation of both lipid and either encapsulated or bound protein after internalization by macrophages (Dijkstra et al., 1984;

Storm et al., 1988; Derksen et al., 1988). Increased release of DOX from liposomes was observed after uptake by peritoneal macrophages, and collected supernatants were shown to have considerable growth-inhibitory activity (Storm et al., 1988). The degradation rate was dependent on the lipid composition of the liposomes, with liposomes containing high-phase transition phospholipids (slow release) being degraded more slowly than those containing low-phase transition phospholipids (rapid release; Storm et al., 1988). However, intracellular processing may vary depending on the cell type and may be significantly different in tumor cells compared with phagocytes such as macrophages. For example, two studies have shown that T cells are able to process liposome-delivered drugs more rapidly than B cells (Machy et al., 1982; Lopes de Menezes et al., 1998). Nevertheless, several other cell types, such as fibroblasts, endothelial cells, and tumor cells, have demonstrated processing of liposomes or their components after internalization (Straubinger et al., 1983; Jett et al., 1985; Trubetskaya et al., 1988; Chu et al., 1990). Internalization of liposomal drugs has also been suggested to increase efficacy by limiting diffusion of the drug away from the cancer cells (Allen et al., 1998). This is especially a concern in the turbulent environment of the general circulation or peritoneal cavity (Allen and Moase, 1996). Both of these factors likely play a role in the increased efficacy observed with actively targeted liposomes. Regardless of the mechanism, targeting to internalizing receptors appears to increase the growth-inhibitory effects of some liposomal drugs.

Methotrexate or other reduced folates are good candidates for delivery via this kind of targeted approach. Due to the relatively low pK^a of the 2′ carboxyl group, methotrexate and its derivatives are not readily protonated and thus cannot passively transverse artificial or biological membranes. The result of this is relatively stable liposome formulations of reduced folates such as methotrexate, which are significantly less likely than even anthracyclines to leak prematurely in the circulation and cause nonspecific toxicities. These reduced folates enter the cell by reduced folate carriers located in the plasma membrane (and membranes of endosomes and lysosomes) of certain cells (Kamen et al., 1991; Westerhof et al., 1995). Reduced folate carriers are upregulated in a variety of different tumor models, as is expected considering the rapid growth rate of cancerous cells (Westerhof et al., 1991; Weitman et al., 1992a; Ross et al., 1994). After the delivery to late endosome and lysosomes where the carrier is degraded and methotrexate is released, methotrexate can be transported by the reduced folate carrier into the cytosol, where it can elicit its cytotoxic action on folate-requiring enzymes (Weitman et al., 1992b; Antony, 1996). A number of in vitro studies with methotrexate or methotrexate-γ-aspartate have shown a marked dependence of cytotoxicity on targeting to endocytic pathways (Heath et al., 1983; Matthay et al., 1986, 1989; Bernstein et al., 1987; Straubinger et al., 1988; Singh et al., 1989). An additional advantage of the development of a liposomal formulation of a drug such as methotrexate is that the modes of drug resistance to methotrexate and anthracyclines are markedly different. Consequently, a combination of targeted liposomal methotrexate with targeted or nontargeted DOX may provide an even greater chance for long-term survival. Of course, this is a technical advantage of the use of antifolates with targeted liposomes. The first and most obvious consideration is that the type of cancer is sensitive to antifolates.

C. Hyperthermia and Thermosensitive Liposomes

Hyperthermia has also been used to increase the bioavailability of liposomal drugs in the tumor area. In addition to simply increasing the amount of liposomes that enter the tumor area (see *IIIC. Hyperthermia and Vascular Permeability Factors for Increasing Vascular Permeability*), hyperthermia makes the distribution of liposomes within the tumor more uniform, increasing the bioavailability of the released drug to cells within the tumor (Kirpotin et al., 1999b). This is similar to the effect seen with HER2-targeted immunoliposomes. Hyperthermia can also be used to increase drug bioavailability via a second mechanism. Liposomes can be rendered thermosensitive by replacing some of the DSPC lipid component with DPPC, resulting in an increased leakage of the encapsulated material (Yatvin et al., 1978; Gaber et al., 1995, 1996; Wu et al., 1997) when heated to 42°C. This effect was found to be dependent on the presence of plasma proteins. At 37°C these liposomes are stable and do not release DOX. However, heating to 42°C for 30 min results in a release of >60% of the encapsulated DOX (Gaber et al., 1995). The combination of hyperthermia and L-DOX appears to be a very promising strategy for the treatment of cancer due to its ability to enhance three important characteristics of liposomal drug delivery: tumor accumulation, intratumoral distribution, and bioavailability. One study has already demonstrated increased therapeutic efficacy for DOX-loaded thermosensitive liposomes used in conjunction with hyperthermia (Huang et al., 1994). Different regimens and treatment schedules are currently being investigated for their effect on efficacy and tolerability. The strategy used here illustrates another important aspect of the optimization of liposomal drug delivery. Currently, it is difficult to resolve even the complex relationships existing between various liposome properties (size, charge, permeability characteristics) and pharmacological factors (dose, route of administration) regulating liposome delivery in vivo. Although these relationships have been the primary focus of this review, the future holds a need for elucidation of the complex processes responsible in vivo for regulating tumor permeability and the movement of liposomes within the tumor after extravasation. The ability to manipulate

these processes will undoubtedly provide a greater avenue for increasing drug bioavailability in vivo for difficult-to-treat solid tumors.

A diagram depicting the accumulation and distribution of liposomes in tumors is given in Fig. 8. After extravasation through large pores in the tumor microvasculature, liposomes accumulate in the tumor interstitium. Here they can release their encapsulated drug slowly, where it can be taken up by neighboring tumor cells. Targeted liposomes can also obtain a deeper tissue distribution after endocytosis or transcytosis of the carrier and thus expose a greater area of the tumor to the drug. In addition, liposomes targeted to endocytic pathways are destabilized by lysosomal enzymes, releasing the drug within the tumor cells, where it can act on intracellular targets.

D. Problems with Highly Hydrophilic Drugs and Bioavailability

As mentioned in *VII. Stability in Plasma and Storage*, it is relatively easy to prepare stable liposome formulations with polar drugs that are unable to permeate membranes. However, the usefulness of these liposomes is more limited due to present limitations in the ability to make these drugs bioavailable at the tumor site. Increased delivery of highly hydrophilic drugs (Chu and Szoka, 1992) or oligonucleotides (Woodle et al., 1997) to the site of action is not sufficient in itself to obtain an enhanced therapeutic effect. On arrival, the drug must both be released by the carrier and be taken up by the cells of interest. Drugs that can be are recognized and transported by plasma membrane transporters, such as ara-C and methotrexate (Plageman et al., 1978; Wiley et al., 1982; Kamen et al., 1991), may be useful if they can be released from the carrier (Allen et al., 1992). In the case of methotrexate, liposome targeting and internalization likely give rise to increased drug release, and thus greater cytotoxicity (Heath et al., 1983; Matthay et al., 1989). After internalization, the drug can be subsequently transported by an internal anion transporter into the cytosol (Kamen et al., 1991).

Several approaches are being studied to improve the bioavailability of this class of drugs. pH sensitive liposomes composed of unsaturated phosphatidylethanolamines and mildly acidic amphiphiles have been the most thoroughly studied (Straubinger et al., 1985; Chu et al., 1990; Litzinger and Huang, 1992). The problem with this approach is that these formulations are readily stabilized by plasma components, which insert into the membrane bilayer and reduce the liposome's sensitivity to pH (Liu and Huang, 1989, 1990; D. C. Drummond and D.-L. Daleke, unpublished observations). Recently, two approaches have been attempted to induce acid-mediated leakage of water-soluble content markers. The first is the development of pH-sensitive lipid-anchored copolymers (Meyer et al., 1998b). Incorporation of these polymers into eggPC/Chol liposomes was shown to result in substantial leakage of a water-soluble content marker (pyranine), when the pH was lowered below 5.5. Unlike other pH-sensitive liposomes, release of the marker is not due to fusion but rather to a collapse of the polymer at the phase transition and subsequent collapse of the liposomes or resulting local defects in the membrane that allow for contents leakage. The second approach is the design of cleaveable PEG-DSPE conjugates (Kirpotin et al., 1996). PEG-DSPE is known to stabilize 1,2-dideoyl-3-*sn*-phosphatidylethanolamine containing membranes and prevent fusion of liposomes (Holland et al., 1996a,b; Basanez et al., 1997). Release of PEG from the surface with a sulfhydryl- or an acid-sensitive trigger gives a fusion-competent liposome, capable of releasing its contents. Programming release of PEG-lipid conjugates from the liposome surface through adjustment of the acyl chain composition has been another mechanism for release of the stabilizing polymer (Holland et al., 1996b; Webb et al., 1998). Some groups have even attempted to destabilize liposomes using enzymes that can cleave peptides or sugars from the liposome surface (Pinnaduwage and Huang, 1988; Pak et al., 1997) or by using pH-sensitive peptides (Parente et al., 1988, 1990). Finally, water-soluble polyanions such as oligonucleotides have been complexed with cationic lipids and then delivered effectively to the nucleus of target cells (Zelphati and Szoka, 1996; Meyer et al., 1998a). Although some progress has been made with these systems in vitro and in cell culture, they are still a considerable way from being useful in an in vivo application.

If this class of drugs is to be used in vivo, it will undoubtedly be in the context of SSLs. To be readily released from the liposome, highly water-soluble compounds will likely require the use of fluid phase liposomes. Although low-phase transition lipids such as 1,2-dideoyl-3-*sn*-phosphatidylethanolamine, eggPC, or POPC can be incorporated into SSLs and still remain long circulating, CLs containing these lipids are rapidly removed from the circulation (see *IID. Effect of Membrane Packing Constraints on Pharmacokinetic Parameters*). Thus, steric stabilization provides more flexibility for the type of drug class that can be delivered to tumors with liposomes.

IX. Conclusions

A. Sterically Stabilized versus Rapid-Release Conventional Liposomal Formulations

In theory, slow-release systems that effectively deliver their drug to tumors and release the drug in the near vicinity of tumor cells are more advantageous, and thus should be more therapeutically efficacious, than a rapid-release system where the drug is released from the carrier to a significant extent while in the circulation. When used at equivalent doses, there are no known instances where DOX-loaded eggPC/Chol liposomes (TLC D-99) were shown to be more efficacious than SSL DOX. How-

ever, by definition, efficacy is not dependent on dose, and at present TLC D-99 can be administered at higher doses than Doxil due to the dose-limiting toxicity of H-F syndrome. In the treatment of patients with metastatic breast cancer, TLC D-99 was shown to have an almost identical response rate as reported for Doxil (Ranson et al., 1997; Harris et al., 1998) but had to be delivered at a dose 70% greater than used for Doxil (75 versus 45 mg/m^2 every 3 weeks). Although both formulations are undoubtedly better than free DOX due to decreased toxicities, better patient compliance, and an increased quality of life, drug delivered via sterically stabilized slow-release systems offers two significant advantages. First, because a comparable therapeutic response requires higher doses of DOX to be administered in the case of TLC D-99, cumulative toxicities such as cardiotoxicity are likely to be higher. In addition, due to significant leakage of the drug in the central compartment, compared with the tumor for DOX, more bioavailable drug likely reaches the heart and other healthy tissues. Initially, the large improvements over free DOX will likely make these differences seem minor in comparison. Nevertheless, as L-DOX becomes more widely accepted and replaces free DOX in treatment regimens, these differences in the new higher limits placed on cumulative doses of L-DOX will become important.

A second advantage of slow-release liposomes is that they are more amenable to active targeting of solid tumors. Because eggPC/Chol liposomes release a large proportion of their contents before reaching the tumor, a significantly reduced advantage would be gained by targeting than would be expected for slow-release liposomes. The use of hyperthermia to increase extravasation of liposomes would also benefit more using slow-release systems, where the increased uptake and distribution of drug-loaded liposomes in the tumor would result in a greater increase in overall tumor drug levels. If the drug is released to a greater extent in the circulation, then the drug takes on the pharmacokinetics of the free drug and would not benefit as substantially from hyperthermia, which alters further the pharmacokinetics of the carrier. If hyperthermia were used in conjunction with thermosensitive liposomes to trigger the release of contents, then heat would be administered after accumulation of the drug in the tumor and would in effect reversibly trigger the transformation of liposomes from a slow-release to a very rapid-release system. In addition, the rapid-release liposomes have reduced circulation lifetimes compared with slow-release liposomes, especially SSLs. Liposomes with longer circulation lifetimes would be expected to benefit more from hyperthermia, which has its effect on increasing passive targeting. Future improvements in liposome design by preparing triggerable liposomes that are slow-release systems whereas in the plasma but revert to rapid-release systems on reaching the tumor will presumably result in the most efficacious formulation.

Finally, efficacy results have not shown thus far a favorable response rate for TLC D-99 over Doxil, and potential remedies are presently under consideration for reducing the severity of H-F syndrome. If these are effective, then dose escalation of Doxil will undoubtedly provide a greater therapeutic response.

B. Conventional and Sterically Stabilized Slow-Release Systems

Small, neutral, and solid CLs for drug delivery appear to be limited in their potential usefulness as a drug delivery vehicle if compared with SSL formulations at a similar dose. The dose-independent clearance kinetics of PEG-modified liposomes provides these carriers with a unique ability to remain in the circulation long enough for a therapeutically relevant concentration of drug to accumulate in tumors but at low enough concentrations to avoid certain nonspecific toxicities. However, as mentioned, H-F syndrome limits the dose of SSL DOX that can be administered and thus CLs (DSPC/Chol) may be administered at a dose high enough to give rise to similar tumor concentrations of drug. One potential problem with CL formulations is that increased circulation times and high intratumoral drug concentrations are dependent on drug-induced toxicity to RES macrophages. This presents a problem that has not been adequately addressed: susceptibility to opportunistic infections. In addition, the requirement of long circulation times on drug-induced toxicity would depend heavily on the drug that is formulated. Some drugs such as mitoxantrone are unable to enhance circulation lifetimes by this approach. For delivery of liposomal drug to solid tumors, slow-release CLs are also limited to the delivery of amphipathic drugs that are able to freely transverse the bilayer. SSLs offer the potential advantage of being able to be modified to increase the bioavailability of a variety of drugs whereas maintaining their long circulation times. Thus, CLs appear to be more limited in applicability to very specific conditions, whereas SSLs appear to be more flexible.

The development of SSLs has shifted the focus of liposome research away from improved CL formulations for long circulation. The question of whether CLs have been truly optimized was raised in *IIC. Effect of Liposome Charge on Pharmacokinetic Parameters*. There is at least some evidence to suggest they have not been. If this is the case, then a careful study of the rate of accumulation in tumors, formulation stability, toxicity, and efficacy will have to be completed to determine whether they can be effectively used as carriers of antineoplastic drugs in vivo. This new generation of CLs may provide a plausible alternative to SSLs if carefully optimized.

C. Visions for Future

Liposomal drugs have been suggested to be the long-awaited "magic bullet" cancer therapy due to their abil-

ity to accumulate selectively in the tumor (Matsumura and Maeda, 1986; Maeda and Matsumura, 1989). However, the problem remains that not all cancers and not all patients respond to the "bullet" equivalently. The drug being delivered by liposomes plays an important role in the response achieved. Multidrug resistance has led to significant obstacles in the ability of standard chemotherapy regimens to cure cancer (Chapman and Powis, 1993; Chabner and Longo, 1996). Many studies have shown that combinations of chemotherapeutic agents with nonoverlapping mechanisms of drug resistance may provide a greater opportunity for treating cancer more effectively. Liposomal drugs have the advantage of continually bombarding the cancer cells with low doses of standard chemotherapy and possible overwhelming drug transporters responsible for pumping drugs such as anthracyclines out of the cell (Richardson and Ryman, 1982; Thierry et al., 1989; Rahman et al., 1992). Even with this possibility in mind, it is unlikely that tumors resistant to DOX will be completely eradicated using only L-DOX. One prominent member of the liposome field of study has continually and quite understandably raised the important question, "When are we ever going to get out of the A's" (Szoka, 1998), referring, of course, to the four most studied liposomal drugs: ara-C, anthracyclines, aminoglycosides, and amphotericin B (a potent antifungal agent). It is an excellent question and one that deserves a considerable amount of thought. Liposomes provide an efficient vehicle for delivery of anticancer agents to tumors, but it will almost certainly be necessary to use combinations of different drugs to provide the most effective treatment. Several studies have addressed this concern (Vaage et al., 1993b; Fonseca et al., 1995; Mitsuyasu et al., 1997; Valero et al., 1999). In one study, combination therapy with low doses of both SSL DOX and SSL-VCR was shown to be more effective in the treatment of MC2 mammary tumors than higher doses of either liposomal drug alone (Vaage et al., 1993b). Other studies have reported the combination of L-DOX with other free drugs (Fonseca et al., 1995; Mitsuyasu et al., 1997; Valero et al., 1999). Additional studies that attempt to encapsulate drugs with nonoverlapping modes of drug resistance and significant activity against a particular form of cancer in liposomes or combine free drugs with nonoverlapping modes of drug resistance with presently developed liposomal drugs are needed and may result in more effective drug regimens for the treatment of a variety of difficult-to-treat cancers.

What liposomal drugs should be developed next? This is a difficult question and is dependent on several different variables; including the type of cancer and its response to a particular drug, the stability of the liposome formulation in the circulation, the ability to make the drug bioavailable at the tumor site, and the mode of drug resistance. There is no one correct answer, and investigators are encouraged to be both creative and thorough in their selection and development of other drug formulations. Although methods for liposome targeting using tumor-specific ligands, for increasing extravasation of liposomes into tumors (hyperthermia) and for increasing the bioavailability of the drug selectively at the tumor site, will in all probability increase the overall therapeutic index of a drug such as L-DOX, there is no doubt an important need to develop liposomal formulations of other drugs. These attempts are currently being made in our laboratory (Kirpotin et al., 1999a) and those of others (Allen et al., 1995b; Chang et al., 1997; Colbern et al., 1998; Embree et al., 1998; Gelmon et al., 1999; Newman et al., 1999; Vaage et al., 1999) with an assortment of well-studied chemotherapeutic agents.

Increasing the efficiency of L-DOX by altering its accumulation in tumor or its distribution within tumors or by increasing its bioavailability selectively within the tumor are important strategies being investigated by many in the field. We have concentrated on two approaches to achieve these goals: local hyperthermia and specific targeting to tumor cell-specific epitopes that internalize on binding. The encouraging preclinical studies with HER2-targeted immunoliposomes are in part a result of the long circulation lifetimes provided by steric stabilization combined with the increased bioavailability resulting from endocytosis of the targeted carrier. Anti-HER2 immunoliposomes are presently being considered (HER2 overexpressing) for the treatment of aggressive forms of breast cancer in clinical trials. In addition, Allen and coworkers have recently shown promising preclinical results with SSL immunoliposomes targeted to B cell malignancies using an anti-CD19 antibody (Lopes de Menezes et al., 1998).

The field of liposomal chemotherapy brings together a broad arena of scientific disciplines, including such varied practices as membrane biophysics, chemistry, biochemistry, cell biology, pharmaceutical technology, tumor physiology, toxicology, and clinical oncology. To be successful, scientific groups in the liposome field need to operate at the interface of these various disciplines and take as many of these practices into consideration when rationally designing a drug-carrier system, including the liposomes described in this review. It is hoped that this review will serve as a focal point from which future improvements in liposome technology can be made and, at the same time, as a reminder of how far we have come. The development of additional liposomal drug formulations should be aided by the advancements of the past, many of which are described in this review, but with the realization that no one formulation is ideal for all classes or even subclasses of drugs. The ability to be creative and to adapt what we have learned thus far will determine the success of this field in the future.

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This review was one of several projects that Dr. Papahadjopoulos was working on at the time of his sudden death in September 1998. Dr. Papahadjopoulos was a pioneer in the field of liposome-mediated drug delivery and an integral part of many major breakthroughs in the liposome field. His contributions started with basic membrane biophysics, leading to an understanding of the basic principles that would allow us to use these artificially prepared membranes as drug carriers, and evolved into the clinic with the development of Doxil, a liposome-based chemotherapeutic. Those contributions intervening are far too numerous to list here, and the full impact of his career on the oncology and liposome fields awaits time's trials. His dedication to the treatment of cancer and, more specifically, breast cancer ensured us all of a greater meaning for our research and for our lives. He remains an inspiration to all of us who remain in his laboratory (The Liposome Research Laboratory) at California Pacific Medical Center and, we are sure, to many others in the field as well.

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New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1)

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ABSTRACT

Background: Assessment of the change in tumour burden is an important feature of the clinical evaluation of cancer therapeutics: both tumour shrinkage (objective response) and disease progression are useful endpoints in clinical trials. Since RECIST was published in 2000, many investigators, cooperative groups, industry and government authorities have adopted these criteria in the assessment of treatment outcomes. However, a number of questions and issues have arisen which have led to the development of a revised RECIST guideline (version 1.1). Evidence for changes, summarised in separate papers in this special issue, has come from assessment of a large data warehouse (>6500 patients), simulation studies and literature reviews.

Highlights of revised RECIST 1.1: Major changes include: *Number of lesions to be assessed:* based on evidence from numerous trial databases merged into a data warehouse for analysis purposes, the number of lesions required to assess tumour burden for response determination has been reduced from a maximum of 10 to a maximum of five total (and from five to two per organ, maximum). *Assessment of pathological lymph* nodes is now incorporated: nodes with a *short* axis of $\geqslant$15 mm are considered measurable and assessable as target lesions. The short axis measurement should be included in the sum of lesions in calculation of tumour response. Nodes that shrink to <10 mm short axis are considered normal. *Confirmation of response* is required for trials with response primary endpoint but is no longer required in randomised studies since the control arm serves as appropriate means of interpretation of data. *Disease progression* is clarified in several aspects: in addition to the previous definition of progression in target disease of 20% increase in sum, a 5 mm absolute increase is now required as well to guard against over calling PD when the total sum is very

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small. Furthermore, there is guidance offered on what constitutes 'unequivocal progression' of non-measurable/non-target disease, a source of confusion in the original RECIST guideline. Finally, a section on detection of new lesions, including the interpretation of FDG-PET scan assessment is included. *Imaging guidance*: the revised RECIST includes a new imaging appendix with updated recommendations on the optimal anatomical assessment of lesions.

Future work: A key question considered by the RECIST Working Group in developing RECIST 1.1 was whether it was appropriate to move from anatomic unidimensional assessment of tumour burden to either volumetric anatomical assessment or to functional assessment with PET or MRI. It was concluded that, at present, there is not sufficient standardisation or evidence to abandon anatomical assessment of tumour burden. The only exception to this is in the use of FDG-PET imaging as an adjunct to determination of progression. As is detailed in the final paper in this special issue, the use of these promising newer approaches requires appropriate clinical validation studies.

1. Background

1.1. History of RECIST criteria

Assessment of the change in tumour burden is an important feature of the clinical evaluation of cancer therapeutics. Both tumour shrinkage (objective response) and time to the development of disease progression are important endpoints in cancer clinical trials. The use of tumour regression as the endpoint for phase II trials screening new agents for evidence of anti-tumour effect is supported by years of evidence suggesting that, for many solid tumours, agents which produce tumour shrinkage in a proportion of patients have a reasonable (albeit imperfect) chance of subsequently demonstrating an improvement in overall survival or other time to event measures in randomised phase III studies (reviewed in [1–4]). At the current time objective response carries with it a body of evidence greater than for any other biomarker supporting its utility as a measure of promising treatment effect in phase II screening trials. Furthermore, at both the phase II and phase III stage of drug development, clinical trials in advanced disease settings are increasingly utilising time to progression (or progression-free survival) as an endpoint upon which efficacy conclusions are drawn, which is also based on anatomical measurement of tumour size.

However, both of these tumour endpoints, objective response and time to disease progression, are useful only if based on widely accepted and readily applied standard criteria based on anatomical tumour burden. In 1981 the World Health Organisation (WHO) first published tumour response criteria, mainly for use in trials where tumour response was the primary endpoint. The WHO criteria introduced the concept of an overall assessment of tumour burden by summing the products of bidimensional lesion measurements and determined response to therapy by evaluation of change from baseline while on treatment.[5] However, in the decades that followed their publication, cooperative groups and pharmaceutical companies that used the WHO criteria often 'modified' them to accommodate new technologies or to address areas that were unclear in the original document. This led to confusion in interpretation of trial results[6] and in fact, the application of varying response criteria was shown to lead to very different conclusions about the efficacy of the same regimen.[7] In response to these problems, an International Working Party was formed in the mid 1990s to standardise and simplify response criteria. New criteria, known as RECIST (Response Evaluation Criteria in Solid Tumours), were published in 2000.[8] Key features of the original RECIST include definitions of minimum size of measurable lesions, instructions on how many lesions to follow (up to 10; a maximum five per organ site), and the use of unidimensional, rather than bidimensional, measures for overall evaluation of tumour burden. These criteria have subsequently been widely adopted by academic institutions, cooperative groups, and industry for trials where the primary endpoints are objective response or progression. In addition, regulatory authorities accept RECIST as an appropriate guideline for these assessments.

1.2. Why update RECIST?

Since RECIST was published in 2000, many investigators have confirmed in prospective analyses the validity of substituting unidimensional for bidimensional (and even three-dimensional)-based criteria (reviewed in [9]). With rare exceptions (e.g. mesothelioma), the use of unidimensional criteria seems to perform well in solid tumour phase II studies.

However, a number of questions and issues have arisen which merit answers and further clarity. Amongst these are whether fewer than 10 lesions can be assessed without affecting the overall assigned response for patients (or the conclusion about activity in trials); how to apply RECIST in randomised phase III trials where progression, not response, is the primary endpoint particularly if not all patients have measurable disease; whether or how to utilise newer imaging technologies such as FDG-PET and MRI; how to handle assessment of lymph nodes; whether response confirmation is truly needed; and, not least, the applicability of RECIST in trials of targeted non-cytotoxic drugs. This revision of the RECIST guidelines includes updates that touch on all these points.

1.3. *Process of RECIST 1.1 development*

The RECIST Working Group, consisting of clinicians with expertise in early drug development from academic research organisations, government and industry, together with imaging specialists and statisticians, has met regularly to set the agenda for an update to RECIST, determine the evidence needed to justify the various changes made, and to review emerging evidence. A critical aspect of the revision process was to create a database of prospectively documented solid tumour measurement data obtained from industry and academic group trials. This database, assembled at the EORTC Data Centre under the leadership of Jan Bogaerts and Patrick Therasse (co-authors of this guideline), consists of >6500 patients with >18,000 target lesions and was utilised to investigate the impact of a variety of questions (e.g. number of target lesions required, the need for response confirmation, and lymph node measurement rules) on response and progression-free survival outcomes. The results of this work, which after evaluation by the RECIST Working Group led to most of the changes in this revised guideline, are reported in detail in a separate paper in this special issue.[10] Larry Schwartz and Robert Ford (also co-authors of this guideline) also provided key databases from which inferences have been made that inform these revisions.[11]

The publication of this revised guideline is believed to be timely since it incorporates changes to simplify, optimise and standardise the assessment of tumour burden in clinical trials. A summary of key changes is found in Appendix I. Because the fundamental approach to assessment remains grounded in the anatomical, rather than functional, assessment of disease, we have elected to name this version RECIST 1.1, rather than 2.0.

1.4. *What about volumetric or functional assessment?*

This raises the question, frequently posed, about whether it is 'time' to move from anatomic unidimensional assessment of tumour burden to either volumetric anatomical assessment *or* to functional assessment (e.g. dynamic contrast enhanced MRI or CT or (18)F-fluorodeoxyglucose positron emission tomographic (FDG-PET) techniques assessing tumour metabolism). As can be seen, the Working Group and particularly those involved in imaging research, did not believe that there is at present sufficient standardisation and widespread availability to recommend adoption of these alternative assessment methods. The only exception to this is in the use of FDG-PET imaging as an adjunct to determination of progression, as described later in this guideline. As detailed in paper in this special issue[12], we believe that the use of these promising newer approaches (which could either *add to* or *substitute for* anatomical assessment as described in RECIST) requires appropriate and rigorous clinical validation studies. This paper by Sargent et al. illustrates the type of data that will be needed to be able to define 'endpoints' for these modalities and how to determine where and when such criteria/modalities can be used to improve the reliability with which truly active new agents are identified and truly inactive new agents are discarded in comparison to RECIST criteria in phase II screening trials. The RECIST Working Group looks forward to such data emerging in the next few years to allow the appropriate changes to the next iteration of the RECIST criteria.

2. Purpose of this guideline

This guideline describes a standard approach to solid tumour measurement and definitions for objective assessment of change in tumour size for use in adult and paediatric cancer clinical trials. It is expected these criteria will be useful in all trials where objective response is the primary study endpoint, as well as in trials where assessment of stable disease, tumour progression or time to progression analyses are undertaken, since all of these outcome measures are based on an assessment of anatomical tumour burden and its change on study. There are no assumptions in this paper about the proportion of patients meeting the criteria for any of these endpoints which will signal that an agent or treatment regimen is active: those definitions are dependent on type of cancer in which a trial is being undertaken and the specific agent(s) under study. Protocols must include appropriate statistical sections which define the efficacy parameters upon which the trial sample size and decision criteria are based. In addition to providing definitions and criteria for assessment of tumour response, this guideline also makes recommendations regarding standard reporting of the results of trials that utilise tumour response as an endpoint.

While these guidelines may be applied in malignant brain tumour studies, there are also separate criteria published for response assessment in that setting.[13] This guideline is *not* intended for use for studies of malignant lymphoma since international guidelines for response assessment in lymphoma are published separately.[14]

Finally, many oncologists in their daily clinical practice follow their patients' malignant disease by means of repeated imaging studies and make decisions about continued therapy on the basis of both objective and symptomatic criteria. It is not intended that these RECIST guidelines play a role in that decision making, except if determined appropriate by the treating oncologist.

3. Measurability of tumour at baseline

3.1. *Definitions*

At baseline, tumour lesions/lymph nodes will be categorised measurable or non-measurable as follows:

3.1.1. *Measurable*

Tumour lesions: Must be accurately measured in at least one dimension (*longest* diameter in the plane of measurement is to be recorded) with a *minimum* size of:

- 10 mm by CT scan (CT scan slice thickness no greater than 5 mm; see Appendix II on imaging guidance).
- 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
- 20 mm by chest X-ray.

Malignant lymph nodes: To be considered pathologically enlarged *and* measurable, a lymph node must be ⩾15 mm in *short* axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the *short* axis will be measured and followed (see Schwartz et al. in this Special Issue[15]). See also notes below on 'Baseline documentation of target and non-target lesions' for information on lymph node measurement.

3.1.2. *Non-measurable*

All other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with ⩾10 to <15 mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

3.1.3. *Special considerations regarding lesion measurability*

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment:

Bone lesions:.

- Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.
- Lytic bone lesions or mixed lytic-blastic lesions, with *identifiable soft tissue components*, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the *soft tissue component* meets the definition of measurability described above.
- Blastic bone lesions are non-measurable.

Cystic lesions:.

- Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.
- 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions with prior local treatment:

- Tumour lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable.

3.2. *Specifications by methods of measurements*

3.2.1. *Measurement of lesions*

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

3.2.2. *Method of assessment*

The same method of assessment and the same technique should be used to characterise each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and ⩾10 mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by colour photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung. See Appendix II for more details.

CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. As is described in Appendix II, when CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). More details concerning the use of both CT and MRI for assessment of objective tumour response evaluation are provided in Appendix II.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next (described in greater detail in Appendix II). If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, laparoscopy: The utilisation of these techniques for objective tumour evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

Tumour markers: Tumour markers *alone* cannot be used to assess *objective* tumour response. If markers are initially above

the upper normal limit, however, they must normalise for a patient to be considered in complete response. Because tumour markers are disease specific, instructions for their measurement should be incorporated into protocols on a disease specific basis. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer), have been published.[16–18] In addition, the Gynecologic Cancer Intergroup has developed CA125 progression criteria which are to be integrated with objective tumour assessment for use in first-line trials in ovarian cancer.[19]

Cytology, histology: These techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumour types such as germ cell tumours, where known residual benign tumours can remain). When effusions are known to be a potential adverse effect of treatment (e.g. with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment can be considered if the measurable tumour has met criteria for response or stable disease in order to differentiate between response (or stable disease) and progressive disease.

4. Tumour response evaluation

4.1. *Assessment of overall tumour burden and measurable disease*

To assess objective response or future progression, it is necessary to estimate the *overall tumour burden at baseline* and use this as a comparator for subsequent measurements. Only patients with measurable disease at baseline should be included in protocols where objective tumour response is the primary endpoint. Measurable disease is defined by the presence of at least one measurable lesion (as detailed above in Section 3). In studies where the primary endpoint is tumour progression (either time to progression or proportion with progression at a fixed date), the protocol must specify if entry is restricted to those with measurable disease or whether patients having non-measurable disease only are also eligible.

4.2. *Baseline documentation of 'target' and 'non-target' lesions*

When more than one measurable lesion is present at baseline all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as *target lesions* and will be recorded and measured at baseline (this means in instances where patients have only one or two organ sites involved a *maximum* of two and four lesions respectively will be recorded). For evidence to support the selection of only five target lesions, see analyses on a large prospective database in the article by Bogaerts et al.[10].

Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to *reproducible repeated measurements*. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. To illustrate this point see the example in Fig. 3 of Appendix II.

Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumour. As noted in Section 3, pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of $\geqslant$15 mm by CT scan. Only the *short* axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumour. Nodal size is normally reported as two dimensions in the plane in which the image is obtained (for CT scan this is almost always the axial plane; for MRI the plane of acquisition may be axial, saggital or coronal). The smaller of these measures is the short axis. For example, an abdominal node which is reported as being 20 mm × 30 mm has a short axis of 20 mm and qualifies as a malignant, measurable node. In this example, 20 mm should be recorded as the node measurement (See also the example in Fig. 4 in Appendix II). All other pathological nodes (those with short axis $\geqslant$10 mm but <15 mm) should be considered non-target lesions. Nodes that have a short axis <10 mm are considered non-pathological and should not be recorded or followed.

A *sum of the diameters* (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the *baseline sum diameters*. If lymph nodes are to be included in the sum, then as noted above, only the *short* axis is added into the sum. The baseline sum diameters will be used as reference to further characterise any objective tumour regression in the measurable dimension of the disease.

All other lesions (or sites of disease) including pathological lymph nodes should be identified as *non-target lesions* and should also be recorded at baseline. Measurements are not required and these lesions should be followed as 'present', 'absent', or in rare cases 'unequivocal progression' (more details to follow). In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the case record form (e.g. 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

4.3. *Response criteria*

This section provides the definitions of the criteria used to determine objective tumour response for target lesions.

4.3.1. *Evaluation of target lesions*

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the *smallest sum on study* (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (*Note:* the appearance of one or more new lesions is also considered progression).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

4.3.2. *Special notes on the assessment of target lesions*

Lymph nodes. Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the nodes regress to below 10 mm on study. This means that when lymph nodes are included as target lesions, the 'sum' of lesions may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of <10 mm. Case report forms or other data collection methods may therefore be designed to have target nodal lesions recorded in a separate section where, in order to qualify for CR, each node must achieve a short axis <10 mm. For PR, SD and PD, the actual short axis measurement of the nodes is to be included in the sum of target lesions.

Target lesions that become 'too small to measure'. While on study, all lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g. 2 mm). However, sometimes lesions or lymph nodes which are recorded as target lesions at baseline become so faint on CT scan that the radiologist may not feel comfortable assigning an exact measure and may report them as being 'too small to measure'. When this occurs it is important that a value be recorded on the case report form. If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned (*Note:* It is less likely that this rule will be used for lymph nodes since they usually have a definable size when normal and are frequently surrounded by fat such as in the retroperitoneum; however, if a lymph node is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned in this circumstance as well). This default value is derived from the 5 mm CT slice thickness (but should not be changed with varying CT slice thickness). The measurement of these lesions is potentially non-reproducible, therefore providing this default value will prevent false responses or progressions based upon measurement error. To reiterate, however, if the radiologist is able to provide an actual measure, that should be recorded, even if it is below 5 mm.

Lesions that split or coalesce on treatment. As noted in Appendix II, when non-nodal lesions 'fragment', the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the 'coalesced lesion'.

4.3.3. *Evaluation of non-target lesions*

This section provides the definitions of the criteria used to determine the tumour response for the group of non-target lesions. While some non-target lesions may actually be measurable, they need not be measured and instead should be assessed only *qualitatively* at the time points specified in the protocol.

Complete Response (CR): Disappearance of all non-target lesions and normalisation of tumour marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumour marker level above the normal limits.

Progressive Disease (PD): *Unequivocal progression* (see comments below) of existing non-target lesions. (*Note:* the appearance of one or more new lesions is also considered progression).

4.3.4. *Special notes on assessment of progression of non-target disease*

The concept of progression of non-target disease requires additional explanation as follows:

When the patient also has measurable disease. In this setting, to achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumour burden has increased sufficiently to merit discontinuation of therapy (see examples in Appendix II and further details below). A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to quality for unequivocal progression status. The designation of overall progression *solely* on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.

When the patient has only non-measurable disease. This circumstance arises in some phase III trials when it is not a criterion of study entry to have measurable disease. The same general concepts apply here as noted above, however, in this instance there is no measurable disease assessment to factor into the interpretation of an increase in non-measurable disease burden. Because worsening in non-target disease cannot be easily quantified (by definition: if all lesions are truly non-measurable) a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e. an increase in tumour burden representing an additional 73% increase in 'volume' (which is equivalent to a 20% increase diameter in a measurable lesion). Examples include an increase in a pleural effusion from 'trace' to 'large', an increase in lymphangitic

disease from localised to widespread, or may be described in protocols as 'sufficient to require a change in therapy'. Some illustrative examples are shown in Figs. 5 and 6 in Appendix II. If 'unequivocal progression' is seen, the patient should be considered to have had overall PD at that point. While it would be ideal to have objective criteria to apply to non-measurable disease, the very nature of that disease makes it impossible to do so, therefore the increase must be substantial.

4.3.5. *New lesions*

The appearance of new malignant lesions denotes disease progression; therefore, some comments on detection of new lesions are important. There are no specific criteria for the identification of new radiographic lesions; however, the finding of a new lesion should be unequivocal: i.e. not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumour (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). This is particularly important when the patient's baseline lesions show partial or complete response. For example, necrosis of a liver lesion may be reported on a CT scan report as a 'new' cystic lesion, which it is not.

A lesion identified on a follow-up study in an anatomical location that was *not* scanned at baseline is considered a new lesion and will indicate disease progression. An example of this is the patient who has visceral disease at baseline and while on study has a CT or MRI brain ordered which reveals metastases. The patient's brain metastases are considered to be evidence of PD even if he/she did not have brain imaging at baseline.

If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.

While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive[1] FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up:
   If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD.
   If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan).
   If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

[1] A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

4.4. *Evaluation of best overall response*

The best overall response is the best response recorded from the start of the study treatment until the end of treatment taking into account any requirement for confirmation. On occasion a response may not be documented until after the end of therapy so protocols should be clear if post-treatment assessments are to be considered in determination of best overall response. Protocols must specify how any new therapy introduced before progression will affect best response designation. The patient's best overall response assignment will depend on the findings of both target and non-target disease and will also take into consideration the appearance of new lesions. Furthermore, depending on the nature of the study and the protocol requirements, it may also require confirmatory measurement (see Section 4.6). Specifically, in non-randomised trials where response is the primary endpoint, confirmation of PR or CR is needed to deem either one the 'best overall response'. This is described further below.

4.4.1. *Time point response*

It is assumed that at each protocol specified time point, a response assessment occurs. Table 1 on the next page provides a summary of the overall response status calculation at each time point for patients who have measurable disease at baseline.

When patients have non-measurable (therefore non-target) disease only, Table 2 is to be used.

4.4.2. *Missing assessments and inevaluable designation*

When no imaging/measurement is done at all at a particular time point, the patient is not evaluable (NE) at that time point. If only a subset of lesion measurements are made at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response. This would be most likely to happen in the case of PD. For example, if a patient had a baseline sum of 50 mm with three measured lesions and at follow-up only two lesions were assessed, but those gave a sum of 80 mm, the patient will have achieved PD status, regardless of the contribution of the missing lesion.

4.4.3. *Best overall response: all time points*

The *best overall response* is determined once all the data for the patient is known.

Best response determination in trials where confirmation of complete or partial response IS NOT required: Best response in these trials is defined as the best response across all time points (for example, a patient who has SD at first assessment, PR at second assessment, and PD on last assessment has a best overall response of PR). When SD is believed to be best response, it must also meet the protocol specified minimum time from baseline. If the minimum time is not met when SD is otherwise the best time point response, the patient's best response depends on the subsequent assessments. For example, a patient who has SD at first assessment, PD at second and does not meet minimum duration for SD, will have a best response of PD. The same patient lost to follow-up after the first SD assessment would be considered inevaluable.

Table 1 – Time point response: patients with target (+/– non-target) disease.

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.

Table 2 – Time point response: patients with non-target disease only.

Non-target lesions	New lesions	Overall response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD[a]
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

CR = complete response, PD = progressive disease, and NE = inevaluable.

a 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.

Best response determination in trials where confirmation of complete or partial response IS required: Complete or partial responses may be claimed only if the criteria for each are met at a subsequent time point as specified in the protocol (generally 4 weeks later). In this circumstance, the best overall response can be interpreted as in Table 3.

4.4.4. *Special notes on response assessment*

When nodal disease is included in the sum of target lesions and the nodes decrease to 'normal' size (<10 mm), they may still have a measurement reported on scans. This measurement should be recorded even though the nodes are normal in order not to overstate progression should it be based on increase in size of the nodes. As noted earlier, this means that patients with CR may not have a total sum of 'zero' on the case report form (CRF).

In trials where confirmation of response is required, repeated 'NE' time point assessments may complicate best response determination. The analysis plan for the trial must address how missing data/assessments will be addressed in determination of response and progression. For example, in most trials it is reasonable to consider a patient with time point responses of PR-NE-PR as a confirmed response.

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as 'symptomatic deterioration'. Every effort should be made to document objective progression even after discontinuation of treatment. Symptomatic deterioration is *not* a descriptor of an objective response: it is a reason for stopping study therapy. The objective response status of such patients is to be determined by evaluation of target and non-target disease as shown in Tables 1–3.

Conditions that define 'early progression, early death and inevaluability' are study specific and should be clearly described in each protocol (depending on treatment duration, treatment periodicity).

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine

Table 3 – Best overall response when confirmation of CR and PR required.

Overall response First time point	Overall response Subsequent time point	BEST overall response
CR	CR	CR
CR	PR	SD, PD or PR[a]
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	NE	SD provided minimum criteria for SD duration met, otherwise NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
PR	NE	SD provided minimum criteria for SD duration met, otherwise NE
NE	NE	NE

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.

a If a CR is truly met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR.

needle aspirate/biopsy) before assigning a status of complete response. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

For equivocal findings of progression (e.g. very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.

4.5. *Frequency of tumour re-evaluation*

Frequency of tumour re-evaluation while on treatment should be protocol specific and adapted to the type and schedule of treatment. However, in the context of phase II studies where the beneficial effect of therapy is not known, follow-up every 6–8 weeks (timed to coincide with the end of a cycle) is reasonable. Smaller or greater time intervals than these could be justified in specific regimens or circumstances. The protocol should specify which organ sites are to be evaluated at baseline (usually those most likely to be involved with metastatic disease for the tumour type under study) and how often evaluations are repeated. Normally, all target and non-target sites are evaluated at each assessment. In selected circumstances certain non-target organs may be evaluated less frequently. For example, bone scans may need to be repeated only when complete response is identified in target disease or when progression in bone is suspected.

After the end of the treatment, the need for repetitive tumour evaluations depends on whether the trial has as a goal the response rate or the time to an event (progression/death). If 'time to an event' (e.g. time to progression, disease-free survival, progression-free survival) is the main endpoint of the study, then routine scheduled re-evaluation of protocol specified sites of disease is warranted. In randomised comparative trials in particular, the scheduled assessments should be performed as identified on a calendar schedule (for example: every 6–8 weeks on treatment or every 3–4 months after treatment) and should not be affected by delays in therapy, drug holidays or any other events that might lead to imbalance in a treatment arm in the timing of disease assessment.

4.6. *Confirmatory measurement/duration of response*

4.6.1. *Confirmation*

In non-randomised trials where response is the primary endpoint, confirmation of PR and CR is required to ensure responses identified are not the result of measurement error. This will also permit appropriate interpretation of results in the context of historical data where response has traditionally required confirmation in such trials (see the paper by Bogaerts et al. in this Special Issue[10]). However, in all other circumstances, i.e. in randomised trials (phase II or III) or studies where stable disease or progression are the primary endpoints, confirmation of response is not required since it will not add value to the interpretation of trial results. However, elimination of the requirement for response confirmation may increase the importance of central review to protect against bias, in particular in studies which are not blinded.

In the case of SD, measurements must have met the SD criteria at least once after study entry at a minimum interval (in general not less than 6–8 weeks) that is defined in the study protocol.

4.6.2. *Duration of overall response*

The duration of overall response is measured from the time measurement criteria are first met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded on study).

The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

4.6.3. *Duration of stable disease*

Stable disease is measured from the start of the treatment (in randomised trials, from date of randomisation) until the criteria for progression are met, taking as reference the *smallest sum on study* (if the baseline sum is the smallest, this is the reference for calculation of PD).

The clinical relevance of the duration of stable disease varies in different studies and diseases. If the proportion of patients achieving stable disease for a minimum period of time is an endpoint of importance in a particular trial, the protocol should specify the minimal time interval required between two measurements for determination of stable disease.

Note: The duration of response and stable disease as well as the progression-free survival are influenced by the frequency of follow-up after baseline evaluation. It is not in the scope of this guideline to define a standard follow-up frequency. The frequency should take into account many parameters including disease types and stages, treatment periodicity and standard practice. However, these limitations of the precision of the measured endpoint should be taken into account if comparisons between trials are to be made.

4.7. *Progression-free survival/proportion progression-free*

4.7.1. *Phase II trials*

This guideline is focused primarily on the use of objective response endpoints for phase II trials. In some circumstances, 'response rate' may not be the optimal method to assess the potential anticancer activity of new agents/regimens. In such cases 'progression-free survival' (PFS) or the 'proportion progression-free' at landmark time points, might be considered appropriate alternatives to provide an initial signal of biologic effect of new agents. It is clear, however, that in an uncontrolled trial, these measures are subject to criticism since an apparently promising observation may be related to biological factors such as patient selection and not the impact of the intervention. Thus, phase II screening trials utilising these endpoints are best designed with a randomised control. Exceptions may exist

where the behaviour patterns of certain cancers are so consistent (and usually consistently poor), that a non-randomised trial is justifiable (see for example van Glabbeke et al.[20]). However, in these cases it will be essential to document with care the basis for estimating the expected PFS or proportion progression-free in the absence of a treatment effect.

4.7.2. *Phase III trials*

Phase III trials in advanced cancers are increasingly designed to evaluate progression-free survival or time to progression as the primary outcome of interest. Assessment of progression is relatively straightforward if the protocol requires all patients to have measurable disease. However, restricting entry to this subset of patients is subject to criticism: it may result in a trial where the results are less likely to be generalisable if, in the disease under study, a substantial proportion of patients would be excluded. Moreover, the restriction to entry will slow recruitment to the study. Increasingly, therefore, trials allow entry of both patients with measurable disease as well as those with non-measurable disease only. In this circumstance, care must be taken to explicitly describe the findings which would qualify for progressive disease for those patients *without* measurable lesions. Furthermore, in this setting, protocols must indicate if the maximum number of recorded target lesions for those patients with measurable disease may be relaxed from five to three (based on the data found in Bogaerts et al.[10] and Moskowitz et al.[11]). As found in the 'special notes on assessment of progression', these guidelines offer recommendations for assessment of progression in this setting. Furthermore, if available, validated tumour marker measures of progression (as has been proposed for ovarian cancer) may be useful to integrate into the definition of progression. Centralised blinded review of imaging studies or of source imaging reports to verify 'unequivocal progression' may be needed if important drug development or drug approval decisions are to be based on the study outcome. Finally, as noted earlier, because the date of progression is subject to ascertainment bias, timing of investigations in study arms should be the same. The article by Dancey et al. in this special issue[21] provides a more detailed discussion of the assessment of progression in randomised trials.

4.8. *Independent review of response and progression*

For trials where *objective response* (CR + PR) is the primary endpoint, and in particular where key drug development decisions are based on the observation of a minimum number of responders, it is recommended that all claimed responses be reviewed by an expert(s) independent of the study. If the study is a randomised trial, ideally reviewers should be blinded to treatment assignment. Simultaneous review of the patients' files and radiological images is the best approach.

Independent review of progression presents some more complex issues: for example, there are statistical problems with the use of central-review-based progression time in place of investigator-based progression time due to the potential introduction of informative censoring when the former precedes the latter. An overview of these factors and other lessons learned from independent review is provided in an article by Ford et al. in this special issue.[22]

4.9. *Reporting best response results*

4.9.1. *Phase II trials*

When response is the primary endpoint, and thus all patients must have measurable disease to enter the trial, all patients included in the study must be accounted for in the report of the results, even if there are major protocol treatment deviations or if they are not evaluable. Each patient will be assigned one of the following categories:

1. Complete response
2. Partial response
3. Stable disease
4. Progression
5. Inevaluable for response: specify reasons (for example: early death, malignant disease; early death, toxicity; tumour assessments not repeated/incomplete; other (specify)).

Normally, all *eligible* patients should be included in the denominator for the calculation of the response rate for phase II trials (in some protocols it will be appropriate to include all treated patients). It is generally preferred that 95% two-sided confidence limits are given for the calculated response rate. Trial conclusions should be based on the response rate for all eligible (or all treated) patients and should *not* be based on a selected 'evaluable' subset.

4.9.2. *Phase III trials*

Response evaluation in phase III trials may be an indicator of the relative anti-tumour activity of the treatments evaluated and is almost always a secondary endpoint. Observed differences in response rate may not predict the clinically relevant therapeutic benefit for the population studied. If objective response is selected as a primary endpoint for a phase III study (only in circumstances where a direct relationship between objective tumour response and a clinically relevant therapeutic benefit can be unambiguously demonstrated for the population studied), the same criteria as those applying to phase II trials should be used and all patients entered should have at least one measurable lesion.

In those many cases where response is a secondary endpoint and not all trial patients have measurable disease, the method for reporting overall best response rates must be pre-specified in the protocol. In practice, response rate may be reported using either an 'intent to treat' analysis (all randomised patients in the denominator) or an analysis where only the subset of patients with measurable disease at baseline are included. The protocol should clearly specify how response results will be reported, including any subset analyses that are planned.

The original version of RECIST suggested that in phase III trials one could write protocols using a 'relaxed' interpretation of the RECIST guidelines (for example, reducing the number of lesions measured) but this should no longer be done since these revised guidelines have been amended in such a way that it is clear how these criteria should be applied for all trials in which anatomical assessment of tumour response or progression are endpoints.

Appendix I. Summary of major changes RECIST 1.0 to RECIST 1.1

	RECIST 1.0	RECIST 1.1	Rationale	Reference in special issue (if applicable)
Minimum size measurable lesions	CT: 10 mm spiral 20 mm non-spiral	CT 10 mm; delete reference to spiral scan	Most scans used have 5 mm or less slice thickness Clearer to give instruction based on slice interval if it is greater than 5 mm	
	Clinical: 20 mm	Clinical: 10 mm (must be measurable with calipers)	Caliper measurement will make this reliable	
	Lymph node: not mentioned	CT: ⩾15 mm short axis for target ⩾10–<15 mm for non-target <10 mm is non-pathological	Since nodes are normal structure need to define pathological enlargement. Short axis is most sensitive	Schwartz et al.[15]
Special considerations on lesion measurability	–	Notes included on bone lesions, cystic lesions	Clarify frequently asked questions	
Overall tumour burden	10 lesions (5 per organ)	5 lesions (2 per organ)	Data warehouse analysis shows no loss of information if lesion number reduced from 10 to 5. A maximum of 2 lesions per organ yields sufficient representation per disease site	Bogaerts et al.[10]
Response criteria target disease	CR lymph node not mentioned	CR lymph nodes must be <10 mm short axis	In keeping with normal size of nodes	Schwartz et al.[15]
	PD 20% increase over smallest sum on study or new lesions	PD 20% increase over smallest sum on study (including baseline if that is smallest) and at least 5 mm increase or new lesions	Clarification that if baseline measurement is smaller than any on study measurement, it is reference against which PD is assessed 5 mm absolute increase to guard against over calling PD when total sum is very small and 20% increase is within measurement error	
Response criteria non-target disease	'unequivocal progression' considered as PD	More detailed description of 'unequivocal progression' to indicate that it should not normally trump target disease status. It must be representative of overall disease status change, not a single lesion increase	Confusion with RECIST 1.0 where some were considering PD if 'increase' in any non-target lesion, even when target disease is stable or responding	
New lesions	–	New section on New lesions	To provide guidance on when a lesion is considered new (and thus PD)	
Overall response	Table integrated target and non-target lesions	Two tables: one integrating target and non-target and the other of non-target only	To account for the fact that RECIST criteria are now being used in trials where PFS is the endpoint and not all patients have measurable (target) disease at baseline	Dancey et al.[21]

		Special notes: How to assess and measure lymph nodes CR in face of residual tissue Discussion of 'equivocal' progression	Frequently asked questions on these topics	
Confirmatory measure	For CR and PR: criteria must be met again 4 weeks after initial documentation	Retain this requirement ONLY for non-randomised trials with primary endpoint of response	Data warehouse shows that response rates rise when confirmation is eliminated, but the only circumstance where this is important is in trials where there is no concurrent comparative control and where this measure is the primary endpoint	Bogaerts et al.[10]
Progression-free survival	General comments only	More specific comments on use of PFS (or proportion progression-free) as phase II endpoint Greater detail on PFS assessment in phase III trials	Increasing use of PFS in phase III trials requires guidance on assessment of PD in patients with non-measurable disease	Dancey et al.[21]
Reporting of response results	9 categories suggested for reporting phase II results	Divided into phase II and phase III 9 categories collapsed into 5 In phase III, guidance given about reporting response	Simplifies reporting and clarifies how to report phase II and III data consistently	
Response in phase III trials	More relaxed guidelines possible if protocol specified	This section removed and referenced in section above: no need to have different criteria for phase II and III	Simplification of response assessment by reducing number of lesions and eliminating need for confirmation in randomised studies where response is not the primary endpoint makes separate 'rules' unnecessary	
Imaging appendix	Appendix I	Appendix II: updated with detailed guidance on use of MRI, PET/CT Other practical guidance included	Evolving use of newer modalities addressed. Enhanced guidance in response to frequent questions and from radiology review experience	
New appendices		Appendix I: comparison of RECIST 1.0 and 1.1 Appendix III: frequently asked questions		

Conflict of interest statement

None declared.

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Appendix II. Specifications for standard anatomical radiological imaging

These protocols for image acquisition of computed tomography (CT) and magnetic resonance imaging (MRI) are recommendations intended for patients on clinical trials where RECIST assessment will be performed. Standardisation of imaging requirements and image acquisition parameters is ideal to allow for optimal comparability of subjects within a study and results between studies. These recommendations are designed to balance optimised image acquisition protocols with techniques that should be feasible to perform globally at imaging facilities in all types of radiology practices. These guidelines are not applicable to functional imaging techniques or volumetric assessment of tumour size.

Scanner quality control is highly recommended and should follow standard manufacturer and facility maintenance schedules using commercial phantoms. It is likely that for RECIST unidimensional measurements this will be adequate to produce reproducible measurements. Imaging quality control for CT includes an analysis of image noise and uniformity and CT number as well as spatial resolution. The frequency of quality control analysis is also variable and should focus on clinically relevant scanning parameters. Dose analysis is always important and the use of imaging should follow the ALARA principle, 'As Low As Reasonably Achievable', which refers to making every reasonable effort to maintain radiation exposures as far below the dose limits as possible.

Specific notes

Chest X-ray measurement of lesions surrounded by pulmonary parenchyma is feasible, but not preferable as the measurement represents a summation of densities. Furthermore, there is poor identification of new lesions within the chest on X-ray as compared with CT. Therefore, measurements of pulmonary parenchymal lesions as well as mediastinal disease are optimally performed with CT of the chest. MRI of the chest should only be performed in extenuating circumstances. Even if IV contrast cannot be administered (for example, in the situation of allergy to contrast), a non-contrast CT of the chest is still preferred over MRI or chest X-ray.

CT scans: CT scans of the chest, abdomen, and pelvis should be contiguous throughout all the anatomic region of interest. As a general rule, the minimum size of a measurable lesion at baseline should be no less than double the slice thickness and also have a minimum size of 10 mm (see below for minimum size when scanners have a slice thickness more than 5 mm). While the precise physics of lesion size and partial volume averaging is complex, lesions smaller than 10 mm may be difficult to accurately and reproducibly measure. While this rule is applicable to baseline scans, as lesions potentially decrease in size at follow-up CT studies, they should still be measured. Lesions which are reported as 'too small to measure' should be assigned a default measurement of 5 mm if they are still visible.

The most critical CT image acquisition parameters for optimal tumour evaluation using RECIST are *anatomic coverage, contrast administration, slice thickness, and reconstruction interval.*

a. *Anatomic coverage*: Optimal anatomic coverage for most solid tumours is the chest, abdomen and pelvis. Coverage should encompass all areas of known predilection for metastases in the disease under evaluation and

should additionally investigate areas that may be involved based on signs and symptoms of individual patients. Because a lesion later identified in a body part not scanned at baseline would be considered as a new lesion representing disease progression, careful consideration should be given to the extent of imaging coverage at baseline and at subsequent follow-up time points. This will enable better consistency not only of tumour measurements but also identification of new disease.

b. *IV contrast administration*: Optimal visualisation and measurement of metastases in solid tumours requires consistent administration (dose and rate) of IV contrast as well as timing of scanning. Typically, most abdominal imaging is performed during the portal venous phase and (optimally) about the same time frame after injection on each examination (see Fig. 1 for impact of different phase of IV contrast on lesion measurement). Most solid tumours may be scanned with a single phase after administration of contrast. While triphasic CT scans are sometimes performed on other types of vascular tumours to improve lesion conspicuity, for consistency and uniformity, we would recommend triphasic CT for hepatocellular and neuroendocrine tumours for which this scanning protocol is generally standard of care, and the improved temporal resolution of the triphasic scan will enhance the radiologists' ability to consistently and reproducibly measure these lesions. The precise dose and rate of IV contrast is dependent upon the CT scanning equipment, CT acquisition protocol, the type of contrast used, the available venous access and the medical condition of the patient. Therefore, the method of administration of intravenous contrast agents is variable. Rather than try to institute rigid rules regarding methods for administering contrast agents and the volume injected, it is appropriate to suggest that an adequate volume of a suitable contrast agent should be given so that the metastases are demonstrated to best effect and a *consistent method* is used on subsequent examinations for any given patient (ideally, this would be specified in the protocol or for an institution). It is very important that the same technique be used at baseline and on follow-up examinations for a given patient. This will greatly enhance the reproducibility of the tumour measurements. If prior to enrolment it is known a patient is not able to undergo CT scans with IV contrast due to allergy or renal insufficiency, the decision as to whether a non-contrast CT or MRI (with or without IV contrast) should be used to evaluate the subject at baseline and follow-up should be guided by the tumour type under investigation and the anatomic location of the disease. For patients who develop contraindications to contrast after baseline contrast CT is done, the decision as to whether non-contrast CT or MRI (enhanced or non-enhanced) should be performed should also be based on the tumour type, anatomic location of the disease and should be optimised to allow for comparison to the prior studies if possible. Each case should be discussed with the radiologist to determine if substitution of these other approaches is possible and, if not, the patient should be considered not evaluable from that point forward. Care must be taken in measurement of target lesions on a different modality and interpretation of non-target disease or new lesions, since the same lesion may appear to have a different size using a new modality (see Fig. 2 for a comparison of CT and MRI of the same lesion). Oral contrast is recommended to help visualise and differentiate structures in the abdomen.

c. *Slice thickness and reconstruction interval*: RECIST measurements may be performed at most clinically obtained slice thicknesses. It is recommended that CT scans be performed at 5 mm contiguous slice thickness or less and indeed this guideline presumes a minimum 5 mm thickness in recommendations for measurable lesion definition. Indeed, variations in slice thickness can have an impact on lesion measurement and on detection of new lesions. However, consideration should also be given for minimising radiation exposure. With these parameters, a minimum 10 mm lesion is considered measurable at baseline. Occasionally, institutions may perform medically acceptable scans at slice thicknesses *greater than* 5 mm. If this occurs, the minimum size of measurable lesions at baseline should be *twice the slice*

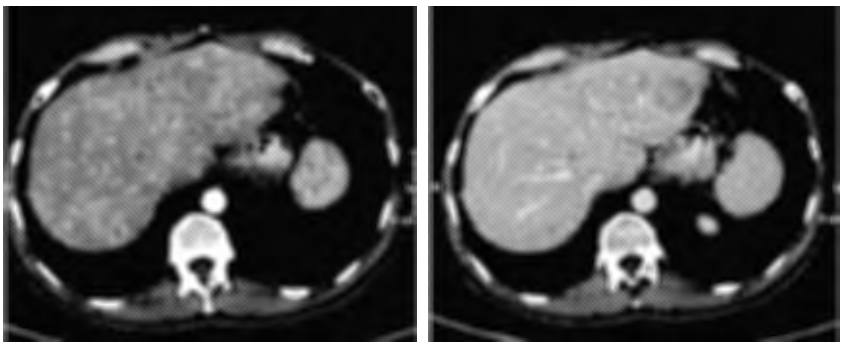

Fig. 1 – Difference in measurement/visualisation with different phases of IV contrast administration. Hypervascular metastases imaged in the arterial phase (left) and the portal venous phase (right). Note that the number of lesions visible differs greatly between the two phases of contrast administration as does any potential lesion measurement. Consistent CT scan acquisition, including phase of contrast administration, is important for optimal and reproducible tumour

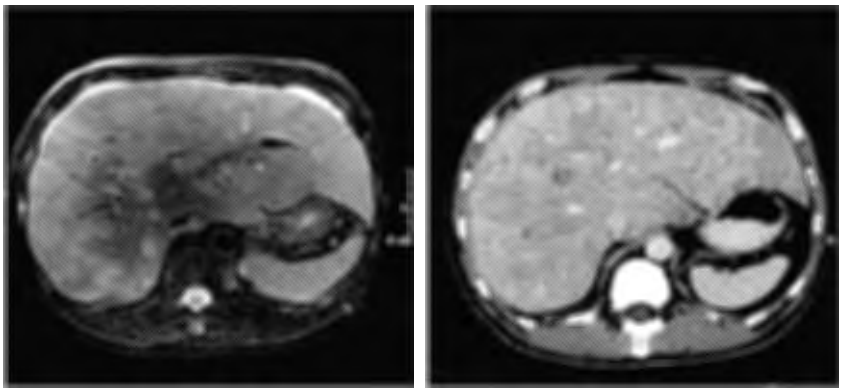

Fig. 2 – CT versus MRI of same lesions showing apparent 'progression' due only to differing method of measurement.

thickness of the baseline scans. Most contemporary CT scanners are multidetector which have many imaging options for these acquisition parameters.[23] The equipment vendor and scanning manual should be reviewed if there are any specific system questions.

d. *Alternative contrast agents*: There are a number of other, new contrast agents, some organ specific.[24] They may be used as part of patient care for instance, in liver lesion assessment, or lymph node characterisation[25], but *should not as yet be used in clinical trials*.

FDG-PET has gained acceptance as a valuable tool for detecting, staging and restaging several malignancies. Criteria for incorporating (or substituting) FDG-PET into anatomical assessment of tumour response in phase II trials are not yet available, though much research is ongoing. Nevertheless, FDG-PET is being used in many drug development trials both as a tool to assess therapeutic efficacy and also in assessment of progression. If FDG-PET scans are included in a protocol, by consensus, an FDG uptake period of 60 min prior to imaging has been decided as the most appropriate for imaging of patients with malignancy.[26] Whole-body acquisition is important since this allows for sampling of all areas of interest and can assess if new lesions have appeared thus determining the possibility of interval progression of disease. Images from the base of the skull to the level of the mid-thigh should be obtained 60 min post injection. PET camera specifications are variable and manufacturer specific, so every attempt should be made to use the same scanner, or the same model scanner, for serial scans on the same patient. Whole-body acquisitions can be performed in either 2- or 3-dimensional mode with attenuation correction, but the method chosen should be consistent across all patients and serial scans in the clinical trial.

PET/CT scans: Combined modality scanning such as with PET–CT is increasingly used in clinical care, and is a modality/technology that is in rapid evolution; therefore, the recommendations in this paper may change rather quickly with time. At present, low dose or attenuation correction CT portions of a combined PET–CT are of limited use in anatomically based efficacy assessments and it is therefore suggested that they should not be substituted for dedicated diagnostic contrast enhanced CT scans for anatomically based RECIST measurements. However, if a site can document that the CT performed as part of a PET–CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast) then the CT portion of the PET–CT can be used for RECIST measurements. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound examinations should not be used in clinical trials to measure tumour regression or progression of lesions because the examination is necessarily subjective and operator dependent. The reasons for this are several: Entire examinations cannot be reproduced for independent review at a later date, and it must be assumed, whether or not it is the case, that the hard-copy films available represent a true and accurate reflection of events. Furthermore, if, for example, the only measurable lesion is in the para-aortic region of the abdomen and if gas in the bowel overlies the lesion, the lesion will not be detected because the ultrasound beam cannot penetrate the gas. Accordingly, the disease staging (or restaging for treatment evaluation) for this patient will not be accurate.

While evaluation of lesions by *physical examination* is also of limited reproducibility, it is permitted when lesions are superficial, at least 10 mm size, and can be assessed using calipers. In general, it is preferred if patients on clinical trials have at least one lesion that is measurable by CT. Other skin or palpable lesions may be measured on physical examination and be considered target lesions.

Use of *MRI* remains a complex issue. MRI has excellent contrast, spatial and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimised for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. Generally, axial imaging of the abdomen and pelvis with T1 and T2 weighted imaging along with gadolinium enhanced imaging should be performed. The field of view, matrix, number of excitations, phase encode steps, use of fat suppression and fast sequences should be optimised for the spe-

cific body part being imaged as well as the scanner utilised. It is beyond the scope of this document or appendix to prescribe specific MRI pulse sequence parameters for all scanners, body parts and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques if possible.

Selection of target lesions: In general, the *largest* lesions representative of involved organs (up to a maximum of two per organ and five total) are selected to follow as target lesions. However, in some cases, the largest lesions may not be easily measured and are not suitable for follow-up because of their configuration. In these cases, identification of the largest *most reproducible* lesions is advised. Fig. 3 provides an illustrative example where the largest lesion is not the most reproducible and another lesion is better to select and follow:

Measurement of lesions

The longest diameter of selected lesions should be measured in the plane in which the images were acquired. For body CT, this is the axial plane. In the event isotropic reconstructions are performed, measurements can be made on these reconstructed images; however, it should be cautioned that not all radiology sites are capable of producing isotropic reconstructions. This could lead to the undesirable situation of measurements in the axial plane at one assessment point and in a different plane at a subsequent assessment. There are some tumours, for instance paraspinal lesions, which are better measured in the coronal or sagittal plane. It would be acceptable to measure these lesions in these planes if the reconstructions in those planes were isotropic or the images were acquired with MRI in those planes. Using the same plane of evaluation, the maximal diameter of each target lesion should always be measured at subsequent follow-up time points even if this results in measuring the lesion at a different slice level or in a different orientation or vector compared with the baseline study. Software tools that calculate the maximal diameter for a perimeter of a tumour may be employed and may even reduce variability.

The only exception to the longest diameter rule is lymph node measurement. Because malignant nodes are identified by the length of their short axis, this is the guide used to determine not only whether they are pathological but is also the dimension measured for adding into the sum of target lesions. Fig. 4 illustrates this point: the large arrow identifies a malignant node: the shorter perpendicular axis is $\geqslant$15 mm and will be recorded. Close by (small arrow) there is a normal node: note here the long axis is greater than 10 mm but the short axis is well below 10 mm. This node should be considered non-pathological.

If a lesion disappears and reappears at a subsequent time point it should continue to be measured. However, the patient's response at the point in time when the lesion reappears will depend upon the status of his/her other lesions. For example, if the patient's tumour had reached a CR status and the lesion reappeared, then the patient would be considered PD at the time of reappearance. In contrast, if the tumour status was a PR or SD and one lesion which had disappeared then reappears, its maximal diameter should be added to the sum of the remaining lesions for a calculated response: in other words, the reappearance of an apparently 'disappeared' single lesion amongst many which remain is not in itself en-

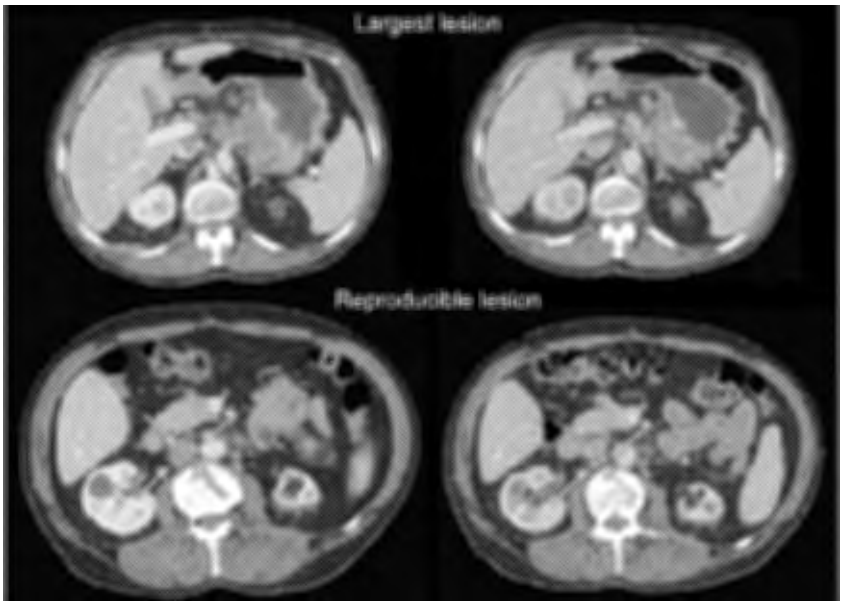

Fig. 3 – Largest lesion may not be most reproducible: most reproducible should be selected as target. In this example, the primary gastric lesion (circled at baseline and at follow-up in the top two images) may be able to be measured with thin section volumetric CT with the same degree of gastric distention at baseline and follow-up. However, this is potentially challenging to reproduce in a multicentre trial and if attempted should be done with careful imaging input and analysis. The most reproducible lesion is a lymph node (circled at baseline and at follow-up in the bottom two images).

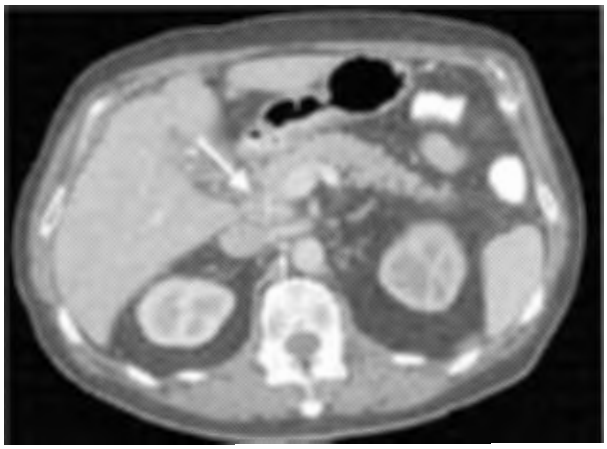

Fig. 4 – Lymph node assessment: large arrow illustrates a pathological node with the short axis shown as a solid line which should be measured and followed. Small arrow illustrates a non-pathological node which has a short axis <10 mm.

ough to qualify for PD: that requires the sum of all lesions to meet the PD criteria. The rationale for such a categorisation is based upon the realisation that most lesions do not actually 'disappear' but are not visualised because they are beyond the resolving power of the imaging modality employed.

The identification of the precise boundary definition of a lesion may be difficult especially when the lesion is embedded in an organ with a similar contrast such as the liver, pancreas, kidney, adrenal or spleen. Additionally, peritumoural oedema may surround a lesion and may be difficult to distinguish on certain modalities between this oedema and actual tumour. In fact, pathologically, the presence of tumour cells within the oedema region is variable. Therefore, it is most critical that the measurements be obtained in a reproducible manner from baseline and all subsequent follow-up time-points. This is also a strong reason to consistently utilise the same imaging modality.

When lesions 'fragment', the individual lesion diameters should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the 'merged lesion'.

Progression of non-target lesions

To achieve 'unequivocal progression' there must be an *overall* level of substantial worsening in non-target disease that is of a magnitude that, even in the presence of SD or PR in target disease, the treating physician would feel it important to change therapy. Examples of unequivocal progression are shown in Figs. 5 and 6.

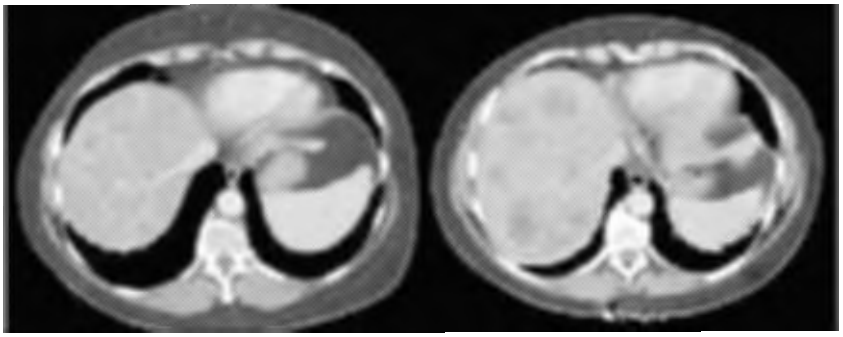

Fig. 5 – Example of unequivocal progression in non-target lesions in liver.

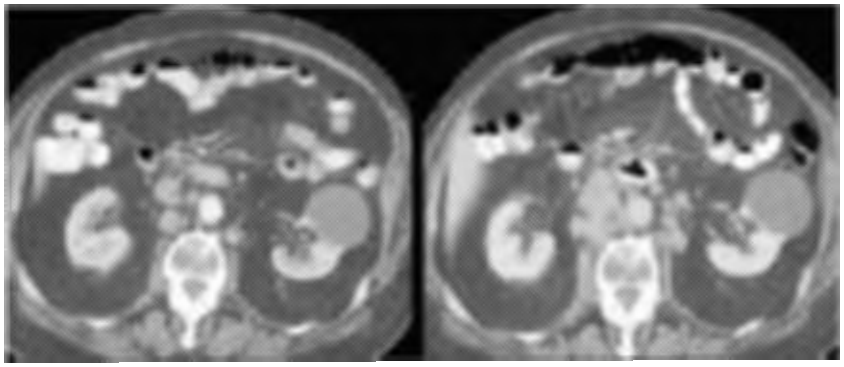

Fig. 6 – Example of unequivocal progression in non-target lesion (nodes).

Appendix III. Frequently asked questions

Question	Answer
What should be done if several unique lesions at baseline become confluent at a follow-up evaluation?	Measure the longest diameter of the confluent mass and record to add into the sum of the longest diameters
How large does a new lesion have to be to count as progression? Does any small subcentimetre lesion qualify, or should the lesion be at least measurable?	New lesions do not need to meet 'measurability criteria' to be considered valid. If it is clear on previous images (with the same technique) that a lesion was absent then its definitive appearance implies progression. If there is any doubt (because of the techniques or conditions) then it is suggested that treatment continue until next scheduled assessment when, generally, all should be clear. Either it gets bigger and the date of progression is the date of the first suspicion, or it disappears and one may then consider it an artefact with the support of the radiologists
How should one lesion be measured if on subsequent exams it is split into two?	Measure the longest diameter of each lesion and add this into the sum
Does the definition of progression depend on the status of all target lesions or only one?	As per the RECIST 1.1 guideline, progression requires a 20% increase in the sum of diameters of all target lesions AND a minimum absolute increase of 5 mm in the sum
Are RECIST criteria accepted by regulatory agencies?	Many cooperative groups and members of pharma were involved in preparing RECIST 1.0 and have adopted them. The FDA was consulted in their development and supports their use, though they don't require it. The European and Canadian regulatory authorities also participated and the RECIST criteria are now integrated in the European note for guidance for the development of anticancer agents. Many pharmaceutical companies are also using them. RECIST 1.1 was similarly widely distributed before publication
What is the criterion for a measurable lesion if the CT slice thickness is >5 mm?	RECIST 1.1 recommends that CT scans have a maximum slice thickness of 5 mm and the minimum size for a measurable lesion is twice that: 10 mm (even if slice thickness is <5 mm). If scanners with slice thickness >5 mm are used, the minimum lesion size must have a longest diameter twice the actual slice thickness
What should we record when target lesions become so small they are below the 10 mm 'measurable' size?	Target lesion measurability is defined at baseline. Thereafter, actual measurements, even if <10 mm, should be recorded. If lesions become very small, some radiologists indicate they are 'too small to measure'. This guideline advises that when this occurs, if the lesion is actually still present, a default measurement of 5 mm should be applied. If in fact the radiologist believes the lesion has gone, a default measurement of 0 mm should be recorded
If a patient has several lesions which have decreased in size to meet PR criteria and one has actually disappeared, does that patient have PD if the 'disappeared' lesion reappears?	Unless the sum meets the PD criteria, the reappearance of a lesion in the setting of PR (or SD) is not PD. The lesion should simply be added into the sum. If the patients had had a CR, clearly reappearance of an absent lesion would qualify for PD
When measuring the longest diameter of target lesions in response to treatment, is the same axis that was used initially used subsequently, even if there is a shape change to the lesion that may have produced a new longest diameter?	The longest diameter of the lesion should always be measured even if the actual axis is different from the one used to measure the lesion initially (or at different time point during follow-up) The only exception to this is lymph nodes: as per RECIST 1.1 the short axis should always be followed and as in the case of target lesions, the vector of the short axis may change on follow-up
Target lesions have been selected at baseline and followed but then one of these target lesions then becomes non-evaluable (i.e. different technique used) What is the effect this has on the other target lesions and the overall response?	What may be done in such cases is one of the following: (a) If the patient is still being treated, call the centre to be sure that future evaluations are done with the baseline technique so at least SOME courses are fully evaluable (b) If that is not possible, check if there IS a baseline exam by the same technique which was used to follow patients...in which case if you retrieve the baseline measures from that technique you retrieve the lesion evaluability (c) If neither (a) nor (b) is possible then it is a judgement call about whether you delete the lesion from all forms or consider the impact of the lesion overall is so important that its being non-evaluable makes the overall response interpretation inevaluable without it. Such a decision should be discussed in a review panel It is NOT recommended that the lesion be included in baseline sums and then excluded from follow-up sums since this biases in favour of a response

(*continued on next page*)

Appendix III – *continued*

Question	Answer
What if a single non-target lesion cannot be reviewed, for whatever reason; does this negate the overall assessment?	Sometimes the major contribution of a single non-target lesion may be in the setting of CR having otherwise been achieved: failure to examine one non-target in that setting will leave you unable to claim CR. It is also possible that the non-target lesion has undergone such substantial progression that it would override the target disease and render patient PD. However, this is very unlikely, especially if the rest of the measurable disease is stable or responding
A patient has a 32% decrease in sum cycle 2, a 28% decrease cycle 4 and a 33% decrease cycle 6. Does confirmation of PR have to take place in sequential scans or is a case like this confirmed PR?	It is not infrequent that tumour shrinkage hovers around the 30% mark. In this case, most would consider PR to have been confirmed looking at this overall case. Had there been two or three non-PR observations between the two time point PR responses, the most conservative approach would be to consider this case SD
In the setting of a breast cancer neoadjuvant study, would mammography not be used to assess lesions? Is CT preferred in this setting?	Neither CT nor mammography are optimal in this setting. MRI is the preferred modality to follow breast lesions in a neoadjuvant setting
A patient has a lesion measurable by clinical exam and by CT scan. Which should be followed?	CT scan. Always follow by imaging if that option exists since it can be reviewed and verified
A lesion which was solid at baseline has become necrotic in the centre. How should this be measured?	The longest diameter of the entire lesion should be followed. Eventually, necrotic lesions which are responding to treatment decrease in size. In reporting the results of trials, you may wish to report on this phenomenon if it is seen frequently since some agents (e.g. angiogenesis inhibitors) may produce this effect
If I am going to use MRI to follow disease, what is minimum size for measurability?	MRI may be substituted for contrast enhanced CT for some sites, but not lung. The minimum size for measurability is the same as for CT (10 mm) as long as the scans are performed with slice thickness of 5 mm and no gap. In the event the MRI is performed with thicker slices, the size of a measurable lesion at baseline should be two times the slice thickness. In the event there are inter-slice gaps, this also needs to be considered in determining the size of measurable lesions at baseline
Can PET–CT be used with RECIST?	At present, the low dose or attenuation correction CT portion of a combined PET–CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if your site has documented that the CT performed as part of a PET–CT is of the same diagnostic quality as a diagnostic CT (with IV and oral contrast) then the PET–CT can be used for RECIST measurements. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed

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ROYAUME UNI

Date
16.02.2018

Reference	Application No./Patent No.
P067376EP:ECO	13731230.2 - 1109 / 2861210
Applicant/Proprietor	
Ipsen Biopharm Ltd.	

Communication of notices of opposition (R. 79(1) EPC)

Notices of opposition have been filed within the opposition period by:

01.
Teva Pharmaceutical Industries Ltd
5 Basel Street
P.O. Box 3190
49131 Petah Tiqva
ISRAEL

The notice of opposition indicated above has already been communicated to you.

You are requested to file your observations within a period of **four months** from notification of this communication.

You may also file amendments, where appropriate, to the description, claims and drawings within the period specified. One set of these documents is to be filed.

If you introduced documents which have not yet been mentioned during the proceedings, your attention is drawn to Rule 83 EPC.

Enclosures:

For the Opposition Division

Europäisches Patentamt • European Patent Office • Office européen des brevets

Registered letter
EPO Form 2317A 12.17 (09/02/18)

Europäisches Patentamt
European Patent Office
Office européen des brevets

Notice of opposition to a European patent

I. Patent opposed

Patent No.	EP2861210
Application No.	EP13731230.2
Date of mention of the grant in the European Patent Bulletin (Art. 97(3), Art. 99(1) EPC)	03 May 2017
Title of the invention	METHODS FOR TREATING PANCREATIC CANCER USING COMBINATION THERAPIES COMPRISING LIPOSOMAL IRINOTECAN

II. Proprietor of the patent

first named in the patent specification	Ipsen Biopharm Ltd.
Opponent's or representative's reference	X112963EP KJG

III. Opponent

Name	Teva Pharmaceutical Industries Ltd
Address:	5 Basel Street PO Box 3190 49131 Petah Tiqva Israel
State of residence or of principal place of business	Israel
Multiple opponents (see additional sheet)	☐

IV. Authorisation

1. Representative

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Multiple representatives (see additional sheet) ☐

Authorisation(s)

is/are enclosed ☐

has/have been registered under No. ☐

V. Opposition is filed against

the patent as a whole ☒

claim(s) No(s).

VI. Grounds for opposition:

Opposition is based on the following grounds:

(a) the subject-matter of the European patent opposed is not patentable (Art. 100(a) EPC) because:

- it is not new (Art. 52(1); Art. 54 EPC) ☒
- it does not involve an inventive step (Art. 52(1); Art. 56 EPC) ☒
- patentability is excluded on other grounds, namely articles ☐

(b) the patent opposed does not disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art (Art. 100(b) EPC; see Art. 83 EPC). ☒

(c) the subject-matter of the patent opposed extends beyond the content of the application/of the earlier application as filed (Art. 100(c) EPC, see Art. 123(2) EPC). ☒

VII. Facts (Rule 76(2)(c) EPC)

presented in support of the opposition are submitted herewith on an attached document ☒

VIII. Other requests:

- Oral proceedings are hereby requested auxiliarily.

IX. Evidence presented

D1	Other evidence	FDA label (Highlights of Prescribing Information) for FUSILEV (levoleucovorin) (2008) original file name: D1 X112963EP.pdf attached as: Other-evidence-1.pdf
D10	Other evidence	FDA label (Highlights of Prescribing Information) for CAMPTOSAR (irinotecan) (2012) original file name: D10 X112963EP.pdf attached as: Other-evidence-10.pdf
D11	Other evidence	Hoskins J M et al., J Natl Cancer Inst (2007) 99:1290-5 original file name: D11 X112963EP.pdf attached as: Other-evidence-11.pdf
D12	Other evidence	Tsai C-S et al., J Gastrointest Oncol (2011) 2(3):185-194 original file name: D12 X112963EP.pdf attached as: Other-evidence-12.pdf
D13	Other evidence	Ko A H et al., J Clin Oncol (2011) 29(15), 4069 original file name: D13 X112963EP.pdf attached as: Other-evidence-13.pdf
D14	Other evidence	T 1409/06 original file name: D14 X112963EP.pdf attached as: Other-evidence-14.pdf
D15	Other evidence	"Study of MM-398 With or Without 5-Fluorouracil and Leucovorin, Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic original file name: D15 X112963EP.pdf attached as: Other-evidence-15.pdf
D16	Other evidence	T 1592/12 original file name: D16 X112963EP.pdf attached as: Other-evidence-16.pdf
D2	Other evidence	Gebbia V et al., Am J Clin Oncol (2010) 33:461-464 original file name: D2 X112963EP.pdf attached as: Other-evidence-2.pdf
D3	Other evidence	Zaniboni A et al., Cancer Chemother Pharmacol (2012) 69:1641-1645 original file name: D3 X112963EP.pdf attached as: Other-evidence-3.pdf
D4	Other evidence	Neuzillet C et al., World J Gastroenterol (September 2012) 18(33):4533-4541 original file name: D4 X112963EP.pdf attached as: Other-evidence-4.pdf
D5	Other evidence	Yoo et al., Br J Cancer (2009) 101:1658-1663 original file name: D5 X112963EP.pdf attached as: Other-evidence-5.pdf
D6	Other evidence	Taïeb J et al., Ann Oncol (2007) 18:498-503 original file name: D6 X112963EP.pdf attached as: Other-evidence-6.pdf

D7	Other evidence	Chen L et al., J Clin Oncol (2008) 26:2565 original file name: D7 X112963EP.pdf attached as: Other-evidence-7.pdf
D8	Other evidence	Infante et al., Cancer Chemother Pharmacol (2012) 70(5), 699 original file name: D8 X112963EP.pdf attached as: Other-evidence-8.pdf
D9	Other evidence	Waterhouse et al., Nanomedicine (2011) 6(9), 1645-1654 original file name: D9 X112963EP.pdf attached as: Other-evidence-9.pdf

X. Payment

Method of payment	Debit from deposit account

The European Patent Office is hereby authorised, to debit from the deposit account with the EPO any fees and costs indicated in the fees section below.

Currency:	EUR
Deposit account number:	28050042
Account holder:	D Young & Co LLP

Refunds

Any refunds should be made to EPO deposit account:	28050042
Account holder:	D Young & Co LLP

Fees	Factor applied	Fee schedule	Amount to be paid
010 Opposition fee	1	785.00	785.00
Total:		EUR	785.00

	A Forms	Details:	System file name:
A-1	Form for notice of opposition		ep-oppo.pdf

	B Attached document files	Original file name:	System file name:
B-1	1. Facts and arguments	Opposition Notice X112963EP.pdf	OPPO.pdf

	C Attached evidence files	Original file name:	System file name:
C-1	1. Other evidence	D1 X112963EP.pdf	Other-evidence-1.pdf
C-2	2. Other evidence	D2 X112963EP.pdf	Other-evidence-2.pdf
C-3	3. Other evidence	D3 X112963EP.pdf	Other-evidence-3.pdf
C-4	4. Other evidence	D4 X112963EP.pdf	Other-evidence-4.pdf
C-5	5. Other evidence	D5 X112963EP.pdf	Other-evidence-5.pdf
C-6	6. Other evidence	D6 X112963EP.pdf	Other-evidence-6.pdf
C-7	7. Other evidence	D7 X112963EP.pdf	Other-evidence-7.pdf

C-8	8. Other evidence	D8 X112963EP.pdf	Other-evidence-8.pdf
C-9	9. Other evidence	D9 X112963EP.pdf	Other-evidence-9.pdf
C-10	10. Other evidence	D10 X112963EP.pdf	Other-evidence-10.pdf
C-11	11. Other evidence	D11 X112963EP.pdf	Other-evidence-11.pdf
C-12	12. Other evidence	D12 X112963EP.pdf	Other-evidence-12.pdf
C-13	13. Other evidence	D13 X112963EP.pdf	Other-evidence-13.pdf
C-14	14. Other evidence	D14 X112963EP.pdf	Other-evidence-14.pdf
C-15	15. Other evidence	D15 X112963EP.pdf	Other-evidence-15.pdf
C-16	16. Other evidence	D16 X112963EP.pdf	Other-evidence-16.pdf

Signature of opponent or representative

Place

Date: **05 February 2018**

Signed by: **Kirk Gallagher 17437**

Association: **D Young & Co LLP**

Representative name: **Kirk Gallagher**

Capacity: **(Representative)**

Annex to the Notice of Opposition against European Patent No. 2861210B1

European Patent Application No. 13731230.2

Ipsen Biopharm Ltd.

Facts and Arguments in accordance with Rule 76(2)(c) EPC

REQUESTS

1. The opponent requests that EP2861210B1 is revoked in its entirety under Articles 100(a), and (b) EPC. In the event that this request is not granted on the basis of the written submissions alone, the opponent requests Oral Proceedings.

EVIDENCE

2. The Opponent relies on the evidence listed in the table below:

D1	FDA label (Highlights of Prescribing Information) for FUSILEV (levoleucovorin) (2008)
D2	Gebbia V et al., *Am J Clin Oncol* (2010) 33:461-464
D3	Zaniboni A et al., *Cancer Chemother Pharmacol* (2012) 69:1641-1645
D4	Neuzillet C et al., *World J Gastroenterol* (September 2012) 18(33):4533-4541
D5	Yoo et al., *Br J Cancer* (2009) 101:1658-1663
D6	Taïeb J et al., *Ann Oncol* (2007) 18:498-503
D7	Chen L et al., *J Clin Oncol* (2008) 26:2565
D8	Infante et al., *Cancer Chemother Pharmacol* (2012) 70(5), 699
D9	Waterhouse et al., *Nanomedicine* (2011) 6(9), 1645-1654
D10	FDA label (Highlights of Prescribing Information) for CAMPTOSAR (irinotecan) (2012)
D11	Hoskins J M et al., *J Natl Cancer Inst* (2007) 99:1290-5
D12	Tsai C-S et al., *J Gastrointest Oncol* (2011) 2(3):185-194
D13	Ko A H et al., *J Clin Oncol* (2011) 29(15), 4069
D14	T 1409/06
D15	"Study of MM-398 With or Without 5-Fluorouracil and Leucovorin, Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer", Clinical Trials Identifier: NCT01494506 (25 January 2013)
D16	T 1592/12

THE PATENT

3. European Patent no. 2861210 ("the Patent") was granted on 3 May 2017, based on application no. 13731230.2. The patent has a filing date of 12 June 2013 and claims priority from two earlier US applications: US 201261659211 P, filed on 13 June 2012; and US 201361784382 P, filed on 14 March 2013.

4. Independent claim 1, is directed to:

"Liposomal irinotecan for use in a method of treating pancreatic cancer in a human patient, wherein the patient exhibits evidence of recurrent or persistent pancreatic cancer following primary chemotherapy and wherein the patient has failed prior treatment with gemcitabine or become resistant to gemcitabine, the method comprising coadministration of an effective amount each of liposomal irinotecan, 5-fluorouracil (5-FU) and leucovorin to the patient in at least one cycle wherein the cycle is a period of 2 weeks and, for each cycle:

(a) liposomal irinotecan is administered to patients not homozygous for the UGT1A1 *28 allele on day 1 of each cycle at a dose of 80 mg/m^2 and to patients homozygous for the UGT1A1 *28 allele on day 1 of cycle 1 at a dose of 60 mg/m^2 and on day 1 of each subsequent cycle at a dose of 60 mg/m^2 or 80 mg/m^2;

(b) 5-FU is administered at a dose of 2400 mg/m^2; and

(c) leucovorin is administered at a dose of 200 mg/m^2 (l form) or 400 mg/m^2 (l + d racemic form);

and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU."

5. Dependent claim 2 further defines the dosing regimen, dependent claim 4 specifies that the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection and dependent claim 5 further specifies the nature of the pancreatic cancer. Claim 3 further defines the administration of the liposomal irinotecan and/or specifies a premedication regimen and/or further specifies the nature of the pancreatic cancer

6. The Patent contains seven examples. Examples 1-4 describe tests with a liposomal irinotecan (MM-398) alone in animal cancer models. Example 5 describes a human pharmacokinetic study with a liposomal irinotecan (MM-398) alone. Example 6 describes a Phase 1 dose escalation study combining a liposomal irinotecan (MM-398), 5-FU and leucovorin in 16 patients with solid tumours, 5 of which had pancreatic cancer. Of the 16 patients in the study, 15 were efficacy evaluable of which 9 patients demonstrated Stable Disease (SD), 4 demonstrated Progressive Disease (PD) and 2 patients had a Partial Response (PR) to the treatment.[1] Neither of the 2 patients demonstrating PR had pancreatic cancer. Of the 5 patients with pancreatic cancer, 1 received 60 mg/m^2, 3

received 80 mg/m^2 and 1 received 120 mg/m^2.[2] No treatment history details (prior gemcitabine therapy) are given for the 5 patients with pancreatic cancer. Moreover, neither the dosing regimen for the drug combination, nor the doses of 5-FU and leucovorin, is provided. Example 7 is prophetic and describes a Phase 3 human study which includes a treatment arm comprising a liposomal irinotecan (MM-398) in combination with 5-FU and leucovorin. There are no results associated with Example 7.

PRIORITY (ARTICLES 87-89 EPC)

7. The Patent is not entitled to the first priority date and hence the effective date of the Patent is 14 March 2013.

8. Claim 1 specifies that leucovorin in racemic form is administered at a dose of 400 mg/m^2, whereas leucovorin in enantiomeric (l-form) is administered at a dose of 200 mg/m^2. However, Claim 3 of the first priority document (PD1) simply states that "...leucovorin is administered at a dose of 200 mg/m^2."

9. The term "leucovorin" without any further definition, as used in PD1, refers to the racemic form of the drug and therefore PD1 describes a different dose from that specified in the granted claims of the Patent.[3] As such, the requirements of Article 87 EPC are not met in respect of the first priority date.

INVENTIVE STEP (ARTICLE 56 EPC)

Background / Introduction

10. At the priority date it was already known to combine irinotecan, 5-FU and leucovorin (FOLFIRI regimen) in the treatment of pancreatic cancer. Thus, document D2 (Gebbia et al) describes a study in 40 patients with advanced pancreatic cancer who had previously been treated with gemcitabine. The patients received irinotecan 180 mg/m^2 followed by levofolinic acid (leucovorin (l-form)) 100 mg/m^2 and 5-FU 1000 mg/m^2, given on a bi-weekly cycle.

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[1] These terms are defined in the patent at [0063]-[0065]
[2] See Table 2; although no details are provided of whether or not the patients are homozygous for the UGT1A1 *28 allele
[3] Enclosed as D1 is the FDA label for the pharmaceutical product FUSILEV which makes clear that leucovorin refers to a mixture of the d- and l- enantiomers; whereas, levoleucovorin is the correct name for the pharmacologically active l-isomer of leucovorin, see section 11 in particular.

11. Similar disclosures of the FOLFIRI regimen can be found in documents D3 (Zaniboni et al), D4 (Neuzillet et al), D5 (Yoo et al), and D6 (Taïeb et al).

12. Further, at the priority date there were several liposomal formulations of irinotecan under development. These liposomal formulations are intended to have improved bioavailability and efficacy compared to conventional irinotecan. Thus, D7 (Chen et al) describes treating patients with advanced refractory solid tumours with PEP02 (MM-398) a nanoparticle liposome formulation of irinotecan. Similarly, D8 (Infante et al)[4] describes IHL-305 a PEGylated liposome containing irinotecan and its use in treating patients with advanced solid tumours, and D9 (Waterhouse et al) describes Irinopore C a further liposomal formulation of irinotecan.

13. At the priority date it was also well-known that Individuals who are homozygous for the UGT1A1*28 allele (UGT1A1 7/7 genotype) are at increased risk for neutropenia following initiation of irinotecan treatment.

14. This is reflected in the FDA labelling information for CAMPTOSAR (irinotecan hydrochloride) in force at the effective date of the patent (see, D10, section 2.3). Similar information is contained in document D11 (Hoskins et al)[5], Abstract and document D8, concerning IHL-305, a liposomal formulation of irinotecan.

Problem-solution analysis

D12 or D13 as closest prior art

15. Ko et al (D12) describes PEP02 (MM-398) as a nanoparticle liposome formulation of irinotecan that has improved pharmacokinetics and tumour bio-distribution compared to the free form of the drug. D12 reports that a combination of PEP02, 5-FU and leucovorin demonstrated prolonged disease control in 5 of 7 (71%) patients with gemcitabine refractory advanced pancreatic cancer.

16. Tsai et al (D13)[6] describes a phase 1 study in seven patients with pancreatic cancer who had failed gemcitabine therapy, see page 189, col. 2, lines 36-42. The patients received a liposomal irinotecan (PEP02 / MM-398) with HDFL (high dose 5-FU / leucovorin). The results of the study suggest the usefulness of the therapy in gemcitabine refractory advanced pancreatic cancer.

[4] Cited as D5 during examination proceedings

Objective technical problem

17. The difference between D12 and D13 and the claimed subject matter is that neither document describes the doses of the active ingredients or the dosing schedule.

18. Thus, the technical problem might be framed as to provide a suitable dosing regimen for the combination therapy described in either D12 or D13

Could / would approach

19. The position of the EPO Boards of Appeal is that although an invention may in theory be based on a dosing regimen the mere determination of the dosage which yields the best effect does not involve an inventive step when the effect (in this case treatment of gemcitabine refractory pancreatic cancer) is already known. The skilled person is aware that the intensity of a pharmacological effect depends *inter alia* on the concentration of the active agent. Finding the optimum dosage is a matter of routine experimentation, which does not require inventive skill, see, T 1409/06; reasons 3.2.1, para, 2 (enclosed as D14).

20. Additionally, the dosing regimen set out in claim 1 of the Patent was already known. D15 describes a phase 3 clinical study of a combination of MM-398, 5-FU and leucovorin in patients with metastatic pancreatic cancer who have failed prior gemcitabine-based therapy. Arm C of the study involves administering 80 mg/m^2 of MM-398, 2400 mg/m^2 of 5-FU and 400 mg/m^2 of leucovorin, every 2 weeks.

21. Thus, starting from either D12 or D13 the claimed subject matter would be arrived at either using routine methods and/or in combination with the information available in D15.

D15 as closest prior art

22. As noted above, D15 describes a phase 3 clinical study of a combination of MM-398, 5-FU and leucovorin in patients with metastatic pancreatic cancer who have failed prior gemcitabine-based therapy. Arm C of the study involves administering 80 mg/m^2 of MM-398, 2400 mg/m^2 of 5-FU and 400 mg/m^2 of leucovorin, every 2 weeks.

23. Given the information in D12 and D13 concerning the efficacy of MM-398, 5-FU and leucovorin in patients with metastatic pancreatic cancer who have failed prior gemcitabine

[5] Cited as D3 during examination proceedings
[6] Cited as D2 during examination proceedings

therapy, it would be obvious to implement the regimen of D15 with a reasonable expectation of success. As such, the claimed subject matter lacks an inventive step.

Dependent claims

24. None of the remaining claims provide any additional features that would render the claims inventive in light of the above analysis. In particular, MM-398 is the liposomal formulation of claim 4. As regards the dosing regimens of claims 2 and 3, no particular technical effect is associated with these features and the dosing regimens would be arrived at using the same common general knowledge of the skilled person referred to above in identifying the dosing regimen of claim 1. Similarly, no distinction can be seen in the pancreatic cancers of the prior art and those described in claim 5.

SUFFICIENCY OF DISCLOSURE (ARTICLE 83 EPC)

25. Article 83 EPC requires that: "The European patent application shall disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art."

26. The requirements of Article 83 EPC must be complied with on the date of filing (Case Law of the Boards of Appeal of the European Patent Office, 8th ed., ("CLBA") at II.C.1 referring to G2/93; page 328 of the English language version). It must be possible to reproduce the invention on the basis of the original application documents without any inventive effort or undue burden, although the skilled person may use his common general knowledge to supplement the information contained in the application (CLBA-II.C.3.1; page 331 of the English language version).

27. With regard to medical use claims, it is well established law that to meet the requirements of Article 83 EPC, the patent/patent application must provide adequate information demonstrating that the treatment is suitably effective in the claimed therapeutic application (CLBA at II.C.6.2; pages 347-349 of the English language version).

28. In broad terms, the Patent claims a drug dosing regimen for treating pancreatic cancer in patients who have failed gemcitabine therapy. The requirements of Article 83 EPC in dosage regimen cases were considered by an EPO Board of Appeal in decision T 1592/12 (Herceptin dosage regimen), enclosed as D16.

29. The patent at issue in decision T 1592/12 related to a Herceptin dosing regimen (8 mg/kg loading dose followed by 6 mg/kg every 3 weeks) for use in treating breast cancer. At the

effective date of the patent at issue, Herceptin was already well-known to be useful in treating breast cancer but the claimed dosing regimen was new. The Board held that it was not enough that Herceptin itself was known to be useful in treating breast cancer, to meet the requirements of Article 83 EPC it was necessary that the patent describes the suitability of the dosing regimen for treating breast cancer:

> "However, the claimed treatment regimen differs from the known treatment regimen in the administration frequency of Herceptin®, *i.e.* every three weeks instead of weekly. From the general principle that the extent of the monopoly conferred by a patent should correspond to, and be justified by, the technical contribution made to the art, it follows that it is the suitability of this different administration frequency to treat breast cancer which needs to be disclosed in the patent for the requirements of sufficiency of disclosure to be met (see also decision T 609/02, *supra*, reasons, point 8)." (Reasons 20)

30. As set out above in paragraph 6, the Patent is devoid of any evidence that the claimed drug dosing regimen is suitable for treating pancreatic cancer in the identified patient group and therefore the requirements of Article 83 EPC are not met.

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use Fusilev safely and effectively. See full prescribing information for Fusilev.

Fusilev (levoleucovorin) INJECTION, POWDER, LYOPHILIZED, FOR SOLUTION for INTRAVENOUS use

Initial U.S. Approval: 1952 (d,l-leucovorin), 2008 (levoleucovorin)

----------INDICATIONS AND USAGE----------

Fusilev is a folate analog. (1)

Fusilev rescue is indicated after high-dose methotrexate therapy in osteosarcoma. (1)

Fusilev is also indicated to diminish the toxicity and counteract the effects of impaired methotrexate elimination and of inadvertent overdosage of folic acid antagonists. (1)

Limitations of Use

Fusilev is not approved for pernicious anemia and megaloblastic anemias. Improper use may cause a hematologic remission while neurologic manifestations continue to progress. (1.1)

----------DOSAGE AND ADMINISTRATION----------

Fusilev Rescue After High-Dose Methotrexate Therapy

Do not administer intrathecally. (2.1)

Fusilev is dosed at one-half the usual dose of the racemic form. (2.1)

Fusilev rescue recommendations are based on a methotrexate dose of 12 grams/m^2 administered by intravenous infusion over 4 hours. Fusilev rescue at a dose of 7.5 mg (approximately 5 mg/m^2) every 6 hours for 10 doses starts 24 hours after the beginning of the methotrexate infusion. Determine serum creatinine and methotrexate levels at least once daily. Continue Fusilev administration, hydration, and urinary alkalinization (pH of 7.0 or greater) until the methotrexate level is below 5 x 10^{-8} M (0.05 micromolar). The Fusilev dose may need to be adjusted. (2.3)

----------DOSAGE FORMS AND STRENGTHS----------

Each 50 mg single-use vial of Fusilev contains a sterile lyophilized powder consisting of 64 mg levoleucovorin calcium pentahydrate (equivalent to 50 mg levoleucovorin) and 50 mg mannitol. (16) It is intended for intravenous administration after reconstitution with 5.3 mL of sterile 0.9% Sodium Chloride for Injection, USP. (2.5, 11)

----------CONTRAINDICATIONS----------

Fusilev is contraindicated for patients who have had previous allergic reactions attributed to folic acid or folinic acid. (4)

----------WARNINGS AND PRECAUTIONS----------

Due to Ca^{++} content, no more than 16 mL (160 mg) of levoleucovorin solution should be injected intravenously per minute. (5.1)

Fusilev enhances the toxicity of fluorouracil. (5.2,7)

Concomitant use of *d,l*-leucovorin with trimethoprim-sulfamethoxazole for Pneumocystis carinii pneumonia in HIV patients was associated with increased rates of treatment failure in a placebo-controlled study. (5.3)

----------ADVERSE REACTIONS----------

Allergic reactions were reported in patients receiving Fusilev. (6.2)

Vomiting (38%), stomatitis (38%) and nausea (19%) were reported in patients receiving Fusilev as rescue after high dose methotrexate therapy. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Spectrum Pharmaceuticals, Inc. at 1-877-387-4538 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch

----------DRUG INTERACTIONS----------

Fusilev may counteract the antiepileptic effect of phenobarbital, phenytoin and primidone, and increase the frequency of seizures in susceptible patients. (7)

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FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

- Fusilev™ is a folate analog.
- Fusilev rescue is indicated after high-dose methotrexate therapy in osteosarcoma.
- Fusilev is also indicated to diminish the toxicity and counteract the effects of impaired methotrexate elimination and of inadvertent overdosage of folic acid antagonists.

1.1 Limitations of Use

- Fusilev is not approved for pernicious anemia and megaloblastic anemias secondary to the lack of vitamin B_{12}. Improper use may cause a hematologic remission while neurologic manifestations continue to progress.

2 DOSAGE AND ADMINISTRATION

2.1 Administration Guidelines

Fusilev is dosed at **one-half** the usual dose of the racemic form.

Fusilev is indicated for intravenous administration only. **Do not administer intrathecally.**

2.2 Co-administration of Fusilev with other agents

Due to the risk of precipitation, do not co-administer Fusilev with other agents in the same admixture.

2.3 Fusilev Rescue After High-Dose Methotrexate Therapy

The recommendations for Fusilev rescue are based on a methotrexate dose of 12 grams/m^2 administered by intravenous infusion over 4 hours (see methotrexate package insert for full prescribing information). Fusilev rescue at a dose of 7.5 mg (approximately 5 mg/m^2) every 6 hours for 10 doses starts 24 hours after the beginning of the methotrexate infusion.

Serum creatinine and methotrexate levels should be determined at least once daily. Fusilev administration, hydration, and urinary alkalinization (pH of 7.0 or greater) should be continued until the methotrexate level is below 5 x 10^{-8} M (0.05 micromolar). The Fusilev dose should be adjusted or rescue extended based on the following guidelines.

Table 1 Guidelines for Fusilev Dosage and Administration

Clinical Situation	Laboratory Findings	Fusilev Dosage and Duration
Normal Methotrexate Elimination	Serum methotrexate level approximately 10 micromolar at 24 hours after administration, 1 micromolar at 48 hours, and less than 0.2 micromolar at 72 hours	7.5 mg IV q 6 hours for 60 hours (10 doses starting at 24 hours after start of methotrexate infusion).
Delayed Late Methotrexate Elimination	Serum methotrexate level remaining above 0.2 micromolar at 72 hours, and more than 0.05 micromolar at 96 hours after administration.	Continue 7.5 mg IV q 6 hours, until methotrexate level is less than 0.05 micromolar.
Delayed Early Methotrexate Elimination and/or Evidence of Acute Renal Injury	Serum methotrexate level of 50 micromolar or more at 24 hours, or 5 micromolar or more at 48 hours after administration, OR; a 100% or greater increase in serum creatinine level at 24 hours after methotrexate administration (e.g., an increase from 0.5 mg/dL to a level of 1 mg/dL or more).	75 mg IV q 3 hours until methotrexate level is less than 1 micromolar; then 7.5 mg IV q 3 hours until methotrexate level is less than 0.05 micromolar.

Patients who experience delayed early methotrexate elimination are likely to develop reversible renal failure. In addition to appropriate Fusilev therapy, these patients require continuing hydration and urinary alkalinization, and close monitoring of fluid and electrolyte status, until the serum methotrexate level has fallen to below 0.05 micromolar and the renal failure has resolved.

Some patients will have abnormalities in methotrexate elimination or renal function following methotrexate administration, which are significant but less severe than the abnormalities described in the table above. These abnormalities may or may not be associated with significant clinical toxicity. If significant clinical toxicity is observed, Fusilev rescue should be extended for an additional 24 hours (total of 14 doses over 84 hours) in subsequent courses of therapy. The possibility that the patient is taking other medications which interact with methotrexate (e.g., medications

which may interfere with methotrexate elimination or binding to serum albumin) should always be reconsidered when laboratory abnormalities or clinical toxicities are observed.

Delayed methotrexate excretion may be caused by accumulation in a third space fluid collection (i.e., ascites, pleural effusion), renal insufficiency, or inadequate hydration. Under such circumstances, higher doses of Fusilev or prolonged administration may be indicated.

Although Fusilev may ameliorate the hematologic toxicity associated with high dose methotrexate, Fusilev has no effect on other established toxicities of methotrexate such as the nephrotoxicity resulting from drug and/or metabolite precipitation in the kidney.

2.4 Dosing Recommendations for Inadvertent Methotrexate Overdosage

Fusilev rescue should begin as soon as possible after an inadvertent overdosage and within 24 hours of methotrexate administration when there is delayed excretion. As the time interval between antifolate administration [e.g., methotrexate] and Fusilev rescue increases, Fusilev's effectiveness in counteracting toxicity may decrease. Fusilev 7.5 mg (approximately 5 mg/m^2) should be administered IV every 6 hours until the serum methotrexate level is less than 10^{-8} M.

Serum creatinine and methotrexate levels should be determined at 24 hour intervals. If the 24 hour serum creatinine has increased 50% over baseline or if the 24 hour methotrexate level is greater than 5×10^{-6} M or the 48 hour level is greater than 9×10^{-7} M, the dose of Fusilev should be increased to 50 mg/m^2 IV every 3 hours until the methotrexate level is less than 10^{-8} M. Hydration (3 L/day) and urinary alkalinization with $NaHCO_3$ should be employed concomitantly. The bicarbonate dose should be adjusted to maintain the urine pH at 7.0 or greater.

2.5 Reconstitution and Infusion Instructions

- Prior to intravenous injection, the 50 mg vial of Fusilev for Injection is reconstituted with 5.3 mL of 0.9% Sodium Chloride Injection, USP to yield a levoleucovorin concentration of 10 mg per mL. Reconstitution with Sodium Chloride solutions with preservatives (e.g. benzyl alcohol) has not been studied. The use of solutions other than 0.9% Sodium Chloride Injection, USP is not recommended.
- The reconstituted 10 mg per mL levoleucovorin contains no preservative. Observe strict aseptic technique during reconstitution of the drug product.
- Saline reconstituted levoleucovorin solutions may be further diluted, immediately, to concentrations of 0.5 mg/mL to 5 mg/mL in 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP. Initial reconstitution or further dilution using 0.9% Sodium Chloride Injection, USP may be held at room temperature for not more than a total of 12 hours. Dilutions in 5% Dextrose Injection, USP may be held at room temperature for not more than 4 hours.
- Visually inspect the reconstituted solution for particulate matter and discoloration, prior to administration. CAUTION: Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use if cloudiness or precipitate is observed.
- No more than 16 mL of reconstituted solutions (160 mg of levoleucovorin) should be injected intravenously per minute, because of the calcium content of the levoleucovorin solution.

3 DOSAGE FORMS AND STRENGTHS

Fusilev is supplied in sterile, single-use vials containing 64 mg levoleucovorin calcium pentahydrate (equivalent to 50 mg levoleucovorin) and 50 mg mannitol.

4 CONTRAINDICATIONS

Fusilev is contraindicated for patients who have had previous allergic reactions attributed to folic acid or folinic acid.

5 WARNINGS AND PRECAUTIONS

5.1 Rate of Administration

Because of the Ca^{++} content of the levoleucovorin solution, no more than 16 mL (160 mg of levoleucovorin) should be injected intravenously per minute.

5.2 Potential for Enhanced Toxicity with 5-Fluorouracil

Fusilev enhances the toxicity of 5-fluorouracil. Deaths from severe enterocolitis, diarrhea, and dehydration have been reported in elderly patients receiving weekly *d,l*-leucovorin and 5-fluorouracil.

5.3 Potential for interaction with trimethoprim-sulfamethoxazole

The concomitant use of *d,l*-leucovorin with trimethoprim-sulfamethoxazole for the acute treatment of Pneumocystis carinii pneumonia in patients with HIV infection was associated with increased rates of treatment failure and morbidity in a placebo-controlled study.

6 ADVERSE REACTIONS

6.1 Clinical Studies Experience

Since clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. The following table presents the frequency of adverse reactions which occurred during the administration of 58 courses of high dose methotrexate 12 grams/m^2 followed by Fusilev rescue for osteosarcoma in 16 patients age 6-21. Most patients received Fusilev 7.5 mg every 6 hours for 60 hours or longer beginning 24 hours after completion of methotrexate.

Table 2 Adverse Reactions

Body System/Adverse Reactions	Number (%) of Patients with Adverse Reactions (N =16)		Number (%) of Courses with Adverse Reactions (N = 58)	
	All	Grade 3+	All	Grade 3+
Gastrointestinal				
Stomatitis	6 (37.5)	1 (6.3)	10 (17.2)	1 (1.7)
Vomiting	6 (37.5)	0	14 (24.1)	0
Nausea	3 (18.8)	0	3 (5.2)	0
Diarrhea	1 (6.3)	0	1 (1.7)	0
Dyspepsia	1 (6.3)	0	1 (1.7)	0
Typhlitis	1 (6.3)	1 (6.3)	1 (1.7)	1 (1.7)
Respiratory				
Dyspnea	1 (6.3)	0	1 (1.7)	0
Skin and Appendages				
Dermatitis	1 (6.3)	0	1 (1.7)	0
Other				
Confusion	1 (6.3)	0	1 (1.7)	0
Neuropathy	1 (6.3)	0	1 (1.7)	0
Renal function abnormal	1 (6.3)	0	3 (5.2)	0
Taste perversion	1 (6.3)	0	1 (1.7)	0
Total number of patients	9 (56.3)		2 (12.5)	
Total number of courses	25 (43.1)		2 (3.4)	

The incidence of adverse reactions may be underestimated because not all patients were fully evaluable for toxicity for all cycles in the clinical trials. Leukopenia and thrombocytopenia were observed, but could not be attributed to high dose methotrexate with Fusilev rescue because patients were receiving other myelosuppressive chemotherapy.

6.2 Postmarketing Experience

Since adverse reactions from spontaneous reports are provided voluntarily from a population of uncertain size, it is not always possible to estimate reliably their frequency or establish a causal relationship to drug exposure. Spontaneously reported adverse reactions collected by the WHO Collaborating Center for International Drug Monitoring in Uppsala Sweden have yielded seven cases where levoleucovorin was administered with a regimen of methotrexate. The events were dyspnea, pruritus, rash, temperature change and rigors. For 217 adverse reactions (108 reports) where levoleucovorin was a suspected or interacting medication, there were 40 occurrences of "possible allergic reaction."

7 DRUG INTERACTIONS

Folic acid in large amounts may counteract the antiepileptic effect of phenobarbital, phenytoin and primidone, and increase the frequency of seizures in susceptible children. It is not known whether folinic acid has the same effects. However, both folic and folinic acids share some common metabolic pathways. Caution should be taken when taking folinic acid in combination with anticonvulsant drugs.

Preliminary human studies have shown that small quantities of systemically administered leucovorin enter the CSF, primarily as its major metabolite, 5-methyltetrahydrofolate (5-MTHFA). In humans, the CSF levels of 5-MTHFA remain 1-3 orders of magnitude lower than the usual methotrexate concentrations following intrathecal administration.

Fusilev increases the toxicity of 5-fluorouracil *[see Warnings and Precautions (5.2)]*.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C. It is not known whether Fusilev can cause fetal harm when administered to a pregnant woman or if it can affect reproduction capacity. Animal reproduction studies have not been conducted with Fusilev. Fusilev should be given to a pregnant woman only if clearly needed.

8.3 Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Fusilev is administered to a nursing mother.

8.4 Pediatric Use

[See Clinical Studies (14)]

8.5 Geriatric Use

Clinical studies of Fusilev in the treatment of osteosarcoma did not include subjects aged 65 and over to determine whether they respond differently from younger subjects.

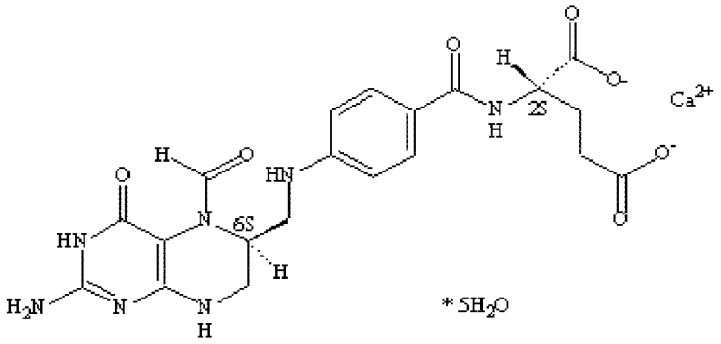
10 OVERDOSAGE

No data are available for overdosage with levoleucovorin.

11 DESCRIPTION

Levoleucovorin is the levo isomeric form of racemic *d,l*-leucovorin, present as the calcium salt. Levoleucovorin is the pharmacologically active isomer of leucovorin [(6-S)-leucovorin].

Fusilev for injection contains levoleucovorin calcium, which is one of several active, chemically reduced derivatives of folic acid. It is useful as antidote to the inhibition of dihydrofolate reductase by methotrexate. This compound has the chemical designation calcium (6S)-N-{4-[[(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl)methyl] amino]benzoyl}-L-glutamate pentahydrate. The molecular weight is 601.6 and the structural formula is:



Its molecular formula is: $C_{20}H_{21}CaN_7O_7 \cdot 5\ H_2O$.

Fusilev for injection is supplied as a sterile lyophilized powder consisting of 64 mg levoleucovorin calcium pentahydrate (equivalent to 50 mg levoleucovorin) and 50 mg mannitol per 50 mg vial.

Sodium hydroxide and/or hydrocholoric acid are used to adjust the pH during manufacture. It is intended for intravenous administration after reconstitution with 5.3 mL of sterile 0.9% Sodium Chloride Injection, USP [*See Dosage and Administration (2.5)*]

12 CLINICAL PHARMACOLOGY

12.1 Mechanism Of Action

Levoleucovorin is the pharmacologically active isomer of 5-formyl tetrahydrofolic acid. Levoleucovorin does not require reduction by the enzyme dihydrofolate reductase in order to participate in reactions utilizing folates as a source of "one-carbon" moieties. Administration of levoleucovorin can counteract the therapeutic and toxic effects of folic acid antagonists such as methotrexate, which act by inhibiting dihydrofolate reductase.

12.2 Pharmacodynamics

Levoleucovorin is actively and passively transported across cell membranes. In vivo, levoleucovorin is converted to 5-methyltetrahydrofolic acid (5-methyl-THF), the primary circulating form of active reduced folate. Levoleucovorin and 5-methyl-THF are polyglutamated intracellularly by the enzyme folylpolyglutamate synthetase. Folylpolyglutamates are active and participate in biochemical pathways that require reduced folate.

12.3 Pharmacokinetics

The pharmacokinetics of levoleucovorin after intravenous administration of a 15 mg dose was studied in healthy male volunteers. After rapid intravenous administration, serum total tetrahydrofolate (total-THF) concentrations reached a mean peak of 1722 ng/mL. Serum (6S)-5-methyl-5,6,7,8-tetrahydrofolate concentrations reached a mean peak of 275 ng/mL and the mean time to peak was 0.9 hours. The mean terminal half-life for total-THF and (6S)-5-methyl-5,6,7,8-tetrahydrofolate was 5.1 and 6.8 hours, respectively.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment Of Fertility

No studies have been conducted to evaluate the potential of levoleucovorin for carcinogenesis, mutagenesis and impairment of fertility.

13.2 Animal Toxicology And/Or Pharmacology

The acute intravenous LD_{50} values in adult mice and rats were 575 mg/kg (1725 mg/m^2) and 378 mg/kg (2268 mg/m^2), respectively. Signs of sedation, tremors, reduced motor activity, prostration, labored breathing, and/or convulsion were

observed in these studies. Anticipated human dose for each administration is approximately 5 mg/m^2, which represents a 3-log safety margin.

14 CLINICAL STUDIES

The safety and efficacy of Fusilev rescue following high-dose methotrexate were evaluated in 16 patients age 6-21 who received 58 courses of therapy for osteogenic sarcoma. High-dose methotrexate was one component of several different combination chemotherapy regimens evaluated across several trials. Methotrexate 12 g/m^2 IV over 4 hours was administered to 13 patients, who received Fusilev 7.5 mg every 6 hours for 60 hours or longer beginning 24 hours after completion of methotrexate. Three patients received methotrexate 12.5 g/m^2 IV over 6 hours, followed by Fusilev 7.5 mg every 3 hours for 18 doses beginning 12 hours after completion of methotrexate. The mean number of Fusilev doses per course was 18.2 and the mean total dose per course was 350 mg. The efficacy of Fusilev rescue following high-dose methotrexate was based on the adverse reaction profile. *[See Adverse Reactions (6)]*

16 HOW SUPPLIED/STORAGE AND HANDLING

Each 50 mg single-use vial of Fusilev for Injection contains a sterile lyophilized powder consisting of 64 mg levoleucovorin calcium pentahydrate (equivalent to 50 mg levoleucovorin) and 50 mg mannitol.

50 mg vial of freeze-dried powder – NDC 68152-101-00.

Store at 25° C (77 °F) in carton until contents are used. Excursions permitted from 15-30° C (59-86 °F). [See USP Controlled Room Temperature]. Protect from light.

SPECTRUM
PHARMACEUTICALS

Manufactured for Spectrum Pharmaceuticals, Inc.
Irvine, CA 92618
Manufactured by Chesapeake Biological Laboratories, Inc.
Baltimore, MD 21230

Spectrum Pharmaceuticals, Inc.

Irinotecan Plus Bolus/Infusional 5-Fluorouracil and Leucovorin in Patients With Pretreated Advanced Pancreatic Carcinoma

A Multicenter Experience of the Gruppo Oncologico Italia Meridionale

Vittorio Gebbia, MD, PhD, Evaristo Maiello, MD,† Francesco Giuliani, MD,‡ Nicolò Borsellino, MD,§ Carlo Arcara, MD,* and Giuseppe Colucci, MD‡*

Background: Patients with advanced pancreatic cancer failing gemcitabine-based first-line chemotherapy are still in relatively good clinical conditions and may still require second-line chemotherapy, which is frequently administered in daily clinical practice given to without solid scientific support.

Patients and Methods: A retrospective survey was carried out including 40 patients with stage III or IV gemcitabine-refractory pancreatic carcinoma. Patients received standard FOLFIRI regimen biweekly until progression or unacceptable toxicity. Response evaluation criteria in solid tumors and National Cancer Institute common toxicity criteria were employed respectively for response and toxicity assessment.

Results: Six partial responses (15%) and 14 stabilizations of disease (35%) were recorded for a tumor growth control rate of 50%. The median time to progression was 3.7 (range, 1–6.5 months), and median overall survival was 6 months (range, 2–8.2 months). A stabilization of performance status and a subjective improvement of cancer-related symptoms were recorded in 21 patients (52.5%). No correlation has been found between length of time to progression during first-line chemotherapy and length of that reported in the second-line setting or objective response. Grade 3–4 diarrhea and mucositis was observed in 15% and 10% of cases, respectively.

Conclusions: Data presented in this article demonstrate that the second-line FOLFIRI regimen are able to induce an objective response in a relatively small fraction of patients with gemcitabine-refractory adenocarcinoma of the pancreas. The use of second-line chemotherapy should be carefully proposed to patients with good performance status or those who had a good response to first-line therapy.

Key Words: FOLFIRI regimen, pancreatic carcinoma, second-line chemotherapy

(*Am J Clin Oncol* 2010;33: 461–464)

In Western countries, pancreatic carcinoma represents the fourth leading cause of death by cancer, with most patients being diagnosed at a locally advanced and/or metastatic stage.[1] Although most studies have reported a 1-year survival rate is lower than 10% for patients with advanced pancreatic carcinoma (APC); however, the availability of gemcitabine (GEM) has represented a small but significant progress in the medical management of advanced disease.[2] In fact, GEM-based chemotherapy represents the standard systemic treatment of APC for the majority of patients, being able to improve cancer-related symptoms and patient's performance status (PS) albeit conferring only a modest survival advantage.[2–4]

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Phase III trials have shown that combination regimens incorporating GEM, cisplatin, 5-fluorouracil (5-FU), oxaliplatin, pemetrexed, or irinotecan (CPT-11) generally improve patient outcomes in terms of objective response rates but with little or no improvement in survival parameters.[3–9] The addition of newer biologic agents, such as marimastat, bevacizumab, or cetuximab, to GEM has not improved the results in terms of survival with an increase in toxicity,[10–12] whereas the addition of erlotinib yielded a very modest improvement in survival as compared with GEM alone.[13] Moreover, the use of sequential chemotherapy, ie, 5-FU plus folinic acid and cisplatin followed by GEM versus the reverse sequence, has also yielded poor results.[14]

In clinical practice, APC patients progressing after GEM-based chemotherapy often are in relatively good clinical conditions and may require a second- and even a third-line therapy. Phase II trials evaluating second-line chemotherapy in patients failing GEM-based chemotherapy are relatively scarce in medical literature. Some agents, such as paclitaxel, oxaliplatin, CPT-11, capecitabine, rubitecan, pemetrexed, and flutamide, have been tested as single agents.[2–4] There are no standard second-line regimens for advanced pancreatic cancer, after gemcitabine failure,[15] even if 2 recently reported studies have indicated that the FOLFOX regimen is superior to both best supportive Care and 5-FU plus folinic acid in the second-line setting.[2,16] Therefore, several off-label drugs shown to be active in advanced APC are employed regularly in daily clinical practice even if no specifically addressed trial has scientifically demonstrated their efficacy in terms of symptoms palliation and survival parameters.

Preclinical studies have shown significant activity of CPT-11 both in cultured pancreatic carcinoma cells and xenograft model with a synergism between if given with 5-FU.[17–20] Single agent CPT-11 has been tested in 2 phase II trials on previously untreated patients with APC yielding response rates of 9% and 27%, respectively.[21,22] In patients pretreated with GEM-based regimens single-agent CPT-11 has been reported to yield a <10% overall response rate with reduction of serum tumor markers in 25% of a small series of patients who showed a 4-months median progression-free survival.[23] A second study employed CPT-11 in combination with raltitrexed with better results.[24]

In this article, we report a retrospective survey of the efficacy and toxicity of CPT-11 in combination with 5-FU and folinic acid (FOLFIRI regimen) administered in the same schedule employed in colon carcinoma in a series of unselected patients affected by APC progressing after GEM-based first-line treatment.[25]

PATIENTS AND METHODS

Patient Population

Patients included in this study showed APC progressing after GEM-based first-line treatment. Patients were enrolled into the study if they satisfied the following inclusion criteria: histologic diagnosis of APC; measurable disease according to the response evaluation criteria in solid tumors[26]; age between 18 and 75 years; Eastern

Cooperative Oncology Group PS ≤2; absence of severe uncontrolled cardiovascular, metabolic, infectious, or neurologic diseases; and informed written consent.[5] Other requirements were adequate bone marrow (platelets count ≥100.000/mm^3, WBC count ≥4000/mm^3, granulocyte count 500/mm^3, a hemoglobin level of ≥10.0 g/mm^3), renal (serum creatinine concentration <2.0 mg/dL), and hepatic functions (serum bilirubin level <2.0 mg/dL and AST 3 or less times the normal level in the absence of liver involvement with cancer or up to 5 times the institutional normal level when cancer was present in the liver).

Pretreatment Evaluation

Staging procedures consisted of medical history, physical examination, Electrocardiogram, peripheral blood cell counts, serum chemistry panel, carcinoembryonic antigen, and CA 19-9. Extent of disease was determined by chest x-rays, computed tomography and/or nuclear magnetic resonance, and endoscopy, as needed. Patients underwent follow-up examinations until death.

Efficacy Assessment

Partial response (PR), stable disease (SD), and progressive disease (PD) were determined according to the response evaluation criteria in solid tumors every 6 cycles.[26] The sum of PR and SD was reported as tumor growth control rate (TGCR). Time to progression (TTP) was estimated from the date of first treatment to the first evidence of PD. Overall survival (OS) was estimated from the date of first cycle of second-line chemotherapy to the date of death or the last follow-up. Clinical benefit assessment was based on patients and physician-reported improvement of cancer-related symptoms and/or stabilization of improvement of PS. This procedure is standard clinical practice at all participating institutions.

Treatment Schedule

The chemotherapy schedules was as follows: CPT-11 180 mg/m^2 on day 1 with levofolinic acid 100 mg/m^2 administered as a 2-hour infusion before 5-FU at 400 mg/m^2 as an i.v. bolus, and 5-FU at 600 mg/m^2 as a 22-hour infusion immediately after 5-FU bolus injection on days 1 and 2.

Treatment was given biweekly until PD or unacceptable toxicity, withdrawal of consent, and physicians decision or treatment interruption for >2 weeks.

Toxicity

Adverse events were graded according to the National Cancer Institute common toxicity criteria (version 3.0). A full blood count was carried out each week to assess hematological toxicity, and the patients had a complete physical examination and serum bilirubin, transaminases, alkaline phosphatase, and creatinine assays before each treatment cycle. The patients were interviewed before each session, focusing on pain, nausea, vomiting, mucositis, diarrhea, asthenia, weight loss, and neurologic disorders. All patients who received at least one treatment session were considered assessable for toxicity. If multiple toxic effects were observed, the dose administered was based on the most severe toxicity experienced. The dose adjustment schedule was evaluated at the beginning of a new administration. Dose reductions were carried out as described previously.[25]

Statistical Analysis

Objective responses were reported as their relative rates adjusted to the nearest unit with 95% confidence interval. TTP and OS were calculated employing a GraphPad statistical software.[27]

RESULTS

Patient Characteristics

As shown in Table 1, 40 patients of 255 screened cases were collected from centers belonging to the Gruppo Oncologico Italia Meridionale (Table 1). Patients were GEM-pretreated and received second-line FOLFIRI regimen between January 2003 and June 2008. There were 24 males (60%) and 16 females (40%) with a median age of 63 years and a median PS of 1, according to the Eastern Cooperative Oncology Group scale. Five patients (12.5%) had a PR to front-line treatment, and 12 patients (30%) had SD. All patients except 7 had metastatic stage IV APC, and 60% had multiple sites of disease. Liver metastases were present in 70% of patients, locoregional lymph nodes in 67.5%, lung metastases in 15%, and peritoneal carcinomatosis in 22.5% of patients.

TABLE 1. Patients Clinical and Demographical Characteristics

No. patients	40 (100%)
Median age (range)	63 (38–73)
Sex	
Male	24 (60%)
Female	16 (40%)
PS (ECOG)	
Median	1
PS 0	2 (0.5%)
PS 1	31 (77%)
PS 2	7 (17.5%)
Stage	
III	7 (17.5%)
IV	33 (82.5%)
Previous surgery	4 (10%)
Previous RT	0
Previous CT	
GEM	22 (55%)
GEMOX	3 (0.7%)
GEM/CDDP	15 (37.5%)
Response to previous chemotherapy	
Partial response	5 (12.5%)
Stable disease	12 (30%)
Progression	23 (57.5%)
Clinical benefit response to previous chemotherapy	
Yes	13 (32.5%)
No	27 (67.5%)
Site of disease	
Pancreas	38 (95%)
Lymph node	27 (67.5%)
Liver	28 (70%)
Peritoneum	9 (22.5%)
Lung	6 (15%)
Other	3 (0.7%)

CDDP indicates cisplatin; CT, chemotherapy; ECOG, Eastern Cooperative Oncology Group; GEM, gemcitabine; GEMOX, gemcitabine and oxaliplatin

Antineoplastic Activity and Survival

As shown in Table 2, there were 6 objective PR (15%), and 14 patients (35%) had an SD for a TGCR of 50%. No complete response was recorded. Median duration of PR was 4.9 months (range, 2–6.5 months). Median TTP from the start of second-line treatment was 3.7 (range, 1–6.5 months). No correlation has been found between length of TTP during first-line chemotherapy and length of TTP in the second-line setting or objective response.

TABLE 2. Activity and Survival Parameters

No. patients	40 (100%)
Partial response	6 (15%)
Stable disease	14 (35%)
Progressive disease	20 (50%)
TGCR	29 (50%)
Clinical benefit	20 (50%)
Median TTP (mo)	3.7 (range, 1–6.5)
GMI (>1.33)	9 (22.5%)
Median OS (mo)	6 (range, 2–8.2)

TABLE 3. Toxicity

Toxicity (NCI-CTC)	Grade 1	Grade 2	Grade 3	Grade 4
Neutropenia	8 (20%)	10 (25%)	4 (10%)	3 (7.5%)
Thrombocytopenia	3 (7.5%)	2 (5%)	1 (2.5%)	0
Anemia	9 (22.5%)	8 (20%)	3 (7.5%)	0
Alopecia	14 (35%)	11 (27.7%)	4 (10%)	0
Diarrhea	13 (32.5%)	10 (25%)	5 (12.5%)	1 (2.5%)
Nausea-vomiting	12 (30%)	9 (22.5%)	3 (7.5%)	0
Mucositis	8 (20%)	8 (20%)	4 (10%)	0
Hand-foot syndrome	3 (7.5%)	0	0	0

NCI-CTC indicates National Cancer Institute common toxicity criteria.

It had been proposed that the activity of a second-line chemotherapy could be documented by showing that the TTP after second-line treatment is longer than the TTP after front-line therapy in each patient. The ratio of TTPs has been defined as the growth modulation index (GMI) with each patient being his own control.[28] A GMI >1 means that TTP was longer with the second-line chemotherapy, and treatment that produces a GMI ≥1.33 (33% improvement) should be considered to have excellent activity.[29] In this series, 9 patients (22.5%) had a GMI >1.33, and 3 further patients had a GMI = 1. Median OS from the start of second-line therapy was 6 months (range, 2–8.2 months). About 21 patients (52.5%) had a stabilization of PS or a subjective improvement of cancer-related symptoms.

Tolerability and Safety

Patients received between 2 and 12 cycles. Most patients received full treatment and median delivered dose intensity was higher than 80% in the whole series. Sixteen patients (40%) had some treatment delay with a median of 3.5 days, but only 3 patients (7.5%) experienced more than 2 cycles of delay. Reasons for delay were not treatment related in 5 cases. Overall, side-effects were moderate and easily manageable with no case of toxic death (Table 3). However, both hematological and gastrointestinal side-effects were commonly observed and managed aggressively, according to the participating institution protocols. Grade 3 anemia was recorded in 7.5% of patients, and grade 3–4 neutropenia occurred in 17.5% of patients with 2 cases of febrile neutropenia, which required hospitalization. G-CSF was employed in 13 cases. Thrombocytopenia occurred in 6 patients (12.5%), but was severe only in 1 case with massive liver disease. Most of the nonhematological symptoms were mild. Grade 3 vomiting occurred in 3 patients (7.5%), even if grade 1–2 vomiting was recorded in 50.5% of cases. Grade 3 mucositis was experienced by 10% of patients, whereas 21 patients had grade 1–2 mucositis. Severe grade 3–4 diarrhea was observed in 6 patients (15%), but mild grade 1–2 diarrhea was quite common, being recorded in more than 50% of cases. Mild hand-foot syndrome was observed in 3 cases (7.5%). When grade 3–4 side-effects are plotted according to PS, 5 of 7 patients with a PS2 had some grade 3 toxicity (71%), whereas grade 3 side-effects were observed in 45% of PS 0–1 patients.

Reasons for treatment discontinuation were progressive cancer in all patients but 3 cases. Two patients had grade 3–4 toxicity, which precluded continuation of chemotherapy, and 1 patient refused to continue for psychologic distress.

DISCUSSION

Despite the disappointing clinical results, systemic chemotherapy has, in the last decade, improved the survival and quality of life of a fraction patients with APC.[30] Systemic chemotherapy with single-agent GEM or a GEM-based regimen still remains a standard of care for the treatment of patients with locally advanced and metastatic pancreatic cancer.[31,32]

To date, there is no defined second-line standard treatment for patients progressing during GEM.[15,31] Several clinical trials have evaluated the efficacy and tolerability of different combination chemotherapy regimens as second-line chemotherapy after GEM failure. Currently available data provide increasing evidence that selected patients with GEM-refractory APC may yield clinical benefit and slight survival improvement from second-line chemotherapy. However, sufficient results from large randomized phase III trials are still lacking and therefore no evidence-based treatment recommendation can be given for patients with APC after failure of first-line GEM.

In this article, we reported the activity and tolerability of the FOLFIRI regimen in a retrospective series of 40 patients with GEM-refractory APC. We reported a 50% TGCR and a 15% PR rate with a median duration of 4.9 months. Median TTP from the start of second-line treatment was 3.7 and median overall survival of 6 months. In this series, 9 patients (22.5%) had a GMI >1.33, and 21 patients (52.5%) had a stabilization of PS or a subjective improvement of cancer-related symptoms. The FOLFIRI regimen has acceptable tolerability, despite grade 3 hematological and gastrointestinal toxicity may occur in up to 18% of cases. Patients with PS2 and/or other factors of poor prognosis may not benefit from this regimen and be exposed to a higher incidence of severe side-effects. Even if no formal comparison has been made, however, the FOLFIRI regimen seems to be associated with higher gastrointestinal side-effects as compared to the toxicity reported in other series of patients with similar characteristics treated with the FOLFOX regimen. On the other hand, antineoplastic data reported in this article, with the FOLFIRI regimen, do not show clinical meaningful differences with results reported by our group in a series of APC patients treated with the FOLFOX regimen.[33] In our hands, the latter regimen achieved a 14% PR rate and a SD in 38% of cases with a median TTP of 4 months and a median OS of 6.7 months. The use of FOLFOX regimen in the second-line treatment of APC patients is supported by 2 studies. Oettle et al compared the FOLFOX regimen with best supportive care in a phase III study on GEM-refractory patients achieving a median overall survival of 21 weeks in the treatment arm compared with 10 weeks in the best supportive care one (P = 0.007) leading to early closure of the study.[34] The CONKO-3 study randomized 168 patients who had GEM-refractory pancreatic cancer to FOLFOX or 5-FU/levofolinic acid.[16] The study was powered at 90% to detect an improved OS by 2 months in the oxaliplatin arm. The median OS of the oxaliplatin arm was 28 weeks as compared with 13 weeks recorded in the 5-FU/levofolinic acid arm, thereby fulfilling the study hypothesis. There was also a significant prolongation of progression-free survival in the FOLFOX arm (13 weeks vs. 9 weeks). Both regimens were tolerable, with the exception of higher neuropathy in the oxaliplatin arm. Therefore, it

has been suggested that the FOLFOX regimen could be regarded as a standard second-line regimen for APC.

In conclusion, data presented in this article support the use of FOLFIRI regimen in the second-line treatment of APC patients. Data from medical literature and our experience support the careful use of second-line chemotherapy in patients with adequate PS or those who had a good response to first-line therapy. Future trials may be needed to validate the role of the FOLFIRI regimen in the second-line treatment of progressing APC.

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ORIGINAL ARTICLE

FOLFIRI as second-line chemotherapy for advanced pancreatic cancer: a GISCAD multicenter phase II study

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Abstract
Purpose The purpose of the present study was to evaluate the activity and the tolerability of the FOLFIRI regimen, administered as second-line chemotherapy in patients with locally advanced or metastatic pancreatic cancer after the failure of a gemcitabine-based regimen.
Methods Patients with locally advanced/metastatic disease who received a first-line chemotherapy (one line only) with gemcitabine ± platinoid (cisplatin, oxaliplatin) and who had measurable disease conform with the RECIST criteria were eligible for the study. FOLFIRI consists of irinotecan 180 mg/m^2 iv on day 1, leucovorin (l-form) 200 mg/m^2 iv on day 1 and 2, 5-FU 400 mg/m^2 iv bolus on days 1 and 2, and 5-FU 600 mg/m^2 iv by ci for 22 h on days 1 and 2, repeated every 2 weeks. The primary end point was the response rate.
Results Among the 50 enrolled patients, 4 partial responses (PR) (8 %) and 14 stable diseases were observed, for a disease control rate of 18/50 (36 %). Forty-one patients (82 %) have been pretreated with cisplatin/oxaliplatin+gemcitabine as first-line chemotherapy. The median progression-free and overall survivals were 3.2 and 5 months, respectively. The 6-month survival rate was 32 %. Grade 3–4 neutropenia and diarrhea occurred in 10 (20 %) and 6 (12 %) patients, respectively.
Conclusion The FOLFIRI regimen showed a modest clinical activity in this quite heavily pretreated patients'

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population with locally advanced or metastatic pancreatic cancer with a manageable toxicity profile.

Keywords Pancreatic cancer · FOLFIRI · Chemotherapy · Second-line · Irinotecan

Introduction

Palliative chemotherapy represents the only treatment option for the vast majority of pancreatic cancer patients, due to the low resectability rate of this deadly disease [1].

Until very recently, gemcitabine monotherapy or its combination with capecitabine or platinoids was considered as standard first-line option, albeit no gemcitabine-based doublet was identified as clearly superior to gemcitabine alone in randomized trials [2–5].

More recently, a new combination based on oxaliplatin, irinotecan, and fluorouracil (FOLFIRINOX) has shown superior results compared to gemcitabine alone and despite its greater toxicity profile is now considered a new first-line standard of treatment, at least in selected patients [6, 7]. After first-line chemotherapy failure, there is no standard second-line therapy of benefit, even if a considerable amount of patients has a good performance status and a relatively low tumor burden [8]. We report here our experience with the FOLFIRI regimen, a potentially non-cross resistant option, as second-line therapy after the failure of a gemcitabine ± a platinum compound-based treatment.

Patients and methods

Patients

Patients with pathologically confirmed, locally advanced, metastatic pancreatic cancer who received a first-line chemotherapy (one line only) with gemcitabine ± platinoid (cisplatin, oxaliplatin) and who had measurable disease conform with the RECIST criteria were eligible for the study.

Other eligibility criteria included: P.S. ECOG 0–1, age ≥18 years and <75 years, at least 4 weeks since completion of any radiation therapy (measurable tumor mass had to be outside the radiation field), and adequate organ function, as indicated by a WBC count ≥3,000/μL, hemoglobin level ≥9 g/dL, platelet count ≥100,000/μL, alkaline phosphatase level ≤5 times the upper limit of normal (ULN), total bilirubin level ≤2 times ULN, serum transaminase level ≤5 times ULN, and creatinine level ≤1.5 mg/dL. Approval of the protocol by each local Independent Bioethical Committee was mandatory, and a written informed consent was required by every enrolled patient.

Treatment

FOLFIRI consists of irinotecan 180 mg/m^2 iv on day 1, leucovorin (l-form) 200 mg/m^2 iv on days 1 and 2, 5-FU 400 mg/m^2 iv bolus on days 1 and 2, 5-FU 600 mg/m^2 iv by ci for 22 h on days 1 and 2 (=one cycle) repeated every 2 weeks.

The use of antiemetic prophylaxis was decided locally.

Patients who developed a severe cholinergic syndrome received preventive treatment with atropine (0.25 mg subcutaneously) during all subsequent cycles.

Patients who developed late-onset diarrhea received high-dose loperamide following specific guidelines.

For any patient with severe toxicity, therapy had to be delayed until complete normalization, and the dose of 5-FU and irinotecan had to be reduced to 80 % of the previous dose for all further administrations.

Palliative and supportive care for the other disease-related symptoms and for toxicity associated with treatment was offered to all subjects.

Treatment consisted of 4 combination-chemotherapy cycles, and in case of stable or responsive disease, other 4 cycles were administered. Further cycles were administered at investigators' discretion for up to 6 months.

Study evaluations

Evaluations before treatment consisted of a complete medical history and physical examination, assessment of performance status, laboratory exams, including hematologic and biochemical tests (within 7 days of study drug start), computed tomography or magnetic resonance imaging of the abdomen or other body areas with disease involvement, and chest X-ray (within 28 days of study drug start).

During treatment, complete physical examination, including performance status and weight, vital sign, and laboratory tests were recorded at each cycle. Radiological assessment for tumor measurement (RECIST 1.1) [9] was done every 4 cycles in the chemotherapy phase until disease progression.

Response criteria and toxicity

The RECIST response criteria were used [9]. A complete response was defined as the disappearance of all measurable and evaluable disease for at least 4 weeks. A partial response (PR) was at least a 30 % decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD. Progressive disease was defined as at least a 20 % increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions. Stable disease (SD) was neither a

sufficient shrinkage to qualify for PR nor a sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started. Survival duration was measured from the initiation of therapy to death or to the last follow-up assessment. Toxicity was graded using the National Cancer Institute Common Toxicity Criteria version 4.0 [23].

Statistical analyses

According to Simon's design (RR not of clinical interest ≤10 %, clinically relevant ≥20 %) [10], 18 consecutive patients were initially treated.

In case of no or one only response achieved, the study should be closed.

In case of >1 response, the accrual should be continued to a total of 42 patients (α 5 %, β 90 %).

Progression-free survival (PFS) was calculated from the start of treatment to the 1st day of progression or drug discontinuation.

Overall survival OS was measured from the 1st day of treatment to the date of death or last follow-up.

OS and PFS were calculated using the Kaplan-Meyer method [11].

Results

Between January 2010 and August 2011, 50 patients were included into the study. After the 42 planned patients as for the Simon's design, 8 additional patients were allowed to enter the study because they were all screened and registered within 10 days from the last (42 nd) patients and before the formal closure notification to all the participating centres. There were 24 male and 26 female patients (female/male ratio, 1:08). Median age was 63 years (range, 47–80). Baseline performance status, according to ECOG, was 0 for 29 patients and 1 for 21 patients.

Level of disease accounted for locally advanced cases in 13 patients (26 %) and metastatic disease in 37 (74 %). Thirty-two out of 37 (86 %) patients with metastatic disease had liver metastases. Primary tumor site was pancreatic head in 34 patients (68 %), body in 13 patients (26 %), and tail in the remaining 3 patients (6 %).

First-line chemotherapy previously administered was GEMOX in 32 patients, CDDP/GEM in 9 patients, GEM alone in 8 patients, and GEM/CAPE in 1 patient.

Chemotherapy regimen was administered for a median of 4 cycles (range, 1–16). Overall response and survival are depicted in Table 1. All the four partial responders had metastatic disease and three out four were responsive to prior GEMOX treatment. Six-month survival was 32 %. Forty-nine patients were available for toxicity assessment.

Toxicity was manageable with a total of 21 patients experiencing at least one episode of grade 3 side effects and 6 patients a grade 4 episode, respectively. One case of febrile neutropenia occurred; no toxic death was reported. The main toxicities are outlined on Table 2.

Discussion

A limited amount of drugs has shown clinical activity and measurable benefits for patients with advanced pancreatic cancer after the failure of a gemcitabine-based first-line chemotherapy [12]. In this setting, irinotecan monotherapy was studied by Yi et al. [13]. Among 33 eligible patients, 3PR and 13 SD were achieved for a DCR of 48 % with manageable toxicity.

Gebbia et al. [14] performed a retrospective analysis in 40 patients with refractory disease recording 6 PR and 14 SD (DCR 50 %) with grade 3–4 diarrhea and mucositis

Table 1 Overall response rate and survival data

	$N = 50$	%	95 % Confidence interval
Partial response	4	8	0.5–15.5
Stable disease	14	28	
Disease control rate (PR+SD)	18	36	22.7–49.3
Progressive disease	26	52	
Not evaluable*	6	12	
PFS (months) median, 3.27; range, 1–11			
OS (months) median, 5.0; range, 1–17			

* Six patients did not complete 4 cycles of treatment due to early progression (5 patients) and consent withdrawal before starting chemotherapy (1 patient). All six are considered as PD in the ITT analysis)

Table 2 Toxicity (G3/G4 grade)

	$N = 49$*	%
Hematologic TOXICITY		
Neutropenia	10 (3)^	20
Thrombocytopenia	1	2
NON-Hematologic TOXICITY		
Asthenia	3 (1)^	6
Alopecia	3	6
Mucositis	2 (1)^	4
Hepatic	5 (2)^	10
Nausea/vomiting	3	6
Diarrhea	6 (2)^	12

* One patient never started chemotherapy

^ Grade 4

Table 3 FOLFIRI regimen in advanced pancreatic cancer

Author	Patients	Pretreatment	IRI dosage	RR (%)	DCR (%)	PFS (months)	OS (months)	6 months OS %	Toxicity (grade 3–4) %
GISCAD (present study)	50	Gemcitabine 8, gemcitabine+oxaliplatin/cisplatin 41, capecitabine/gemcitabine 1	180 mg/m^2 q2wks	8	32	3.2	5.0	32	55
Yoo [15]	31	Gemcitabine 4, gemcitabine/capecitabine 20, gemcitabine/cisplatin 3, gemcitabine/erlotinib 4	70 mg/m^2 d1,3 q2wks	0	23	2.1	4.2	27	38
Neuzill [18]*	70	Gemcitabine/platinum based	180 mg/m^2 100 mg/m^2 d1,3 q2wks	8	43	5.8	6	24 % (12 months)	25.7
Gebbia [14]*	40	Gemcitabine only	180 mg/m^2 q2wks	15	50	3.7	6	NS	25
Taieb [16]	40	Only 1 patient chemo-pretreated	90 mg/m^2 d1,3 q2wks	45	37.5	5.6	12.1	NS	Neutropenia 35 %
Cereda [17]	34	Gemcitabine 34	180 mg/m^2 q2wks	NS	12	2	4.2	NS	Neutropenia 9 %

* Retrospective studies, *NS* = not stated

experienced by 15 and 10 % of patients, respectively. A modified FOLFIRI regimen (FOLFIRI.3) was studied by Yoo et al. [15] in a randomized phase II study versus a modified Folfox regimen. Among the 31 patients treated with the FOLFIRI.3 regimen, a DCR of 23 % and a 6-month survival of 27 % were achieved. Similar encouraging results were reported, in first-line treatment, with a little more intensive version of FOLFIRI.3 by Taieb et al. [16]. On the contrary, Cereda et al. [17] reported substantially negative results with the FOLFIRI or XELIRI regimens in 34 gemcitabine-resistant patients, with 4 SD only and a median survival of 4 months. Finally, a large retrospective French study was recently presented, involving 70 patients previously treated with gemcitabine- and platinum-based chemotherapies, 60 received FOLFIRII, and 10 FOLFIRI.3. A DCR of 44 % was obtained with mild toxicity. one-year survival was 17 % [18].

Our results, obtained in a multicenter community setting, seem to further support the role of the combination of irinotecan and fluorouracil as a second-line option for previously treated pancreatic cancer. Toxicity was manageable with no toxic death reported. Our results seem in line with other similar experience with irinotecan and fluorouracil-based regimens as summarized in Table 3. Even if we have not reached our planned goal of a response rate of at least 10 %, it should be outlined that 41 out of 50 patients (82 %) in our study were pretreated with a gemcitabine+platinoids regimen and only a minority received gemcitabine alone, as allowed in our entry criteria. The lack of cross-resistance between gemcitabine/platinoids combinations and FOLFIRI further outlines its potential role as preferred second-line treatment in this patient population.

In our study, one out of three patients benefitted from the FOLFIRI treatment with mild toxicity. However, for patients pretreated with gemcitabine only, combinations of oxaliplatin and fluoropyrimidine still represent an equally reasonable option [19, 20].

Two potential limitations of our study are represented by the inclusion of locally advanced disease along with metastatic patients. Nowadays, separate trials for these two distinct populations are preferred. A second point of weakness is the lack of a quality of life assessment, in order to better define the palliative effect of the FOLFIRI regimen.

It is clear that little progress has been achieved so far in the second-line treatment of pancreatic cancer. Nevertheless, novel agents with innovative mechanism of action are just around the corner like nab-paclitaxel [21] and newer target therapies as mek inhibitors [22].

These drugs will probably help us in transforming advanced pancreatic cancer into a chronic disease, which remains at the moment one of the more important challenge for modern oncology.

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Conflict of interest None.

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BRIEF ARTICLE

FOLFIRI regimen in metastatic pancreatic adenocarcinoma resistant to gemcitabine and platinum-salts

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Abstract

AIM: To evaluate the efficacy and safety of the FOLFIRI regimen in patients with metastatic pancreatic adenocarcinoma (PAC) after the failure of gemcitabine and platinum salts.

METHODS: All consecutive patients with histologically confirmed, metastatic PAC and World Health Organization performance status (PS) $\leq$ 2 received FOLFIRI-1 [irinotecan 180 mg/m^2 on day 1 and leucovorin 400 mg/m^2 followed by 5-fluorouracil (5-FU) 400 mg/m^2 bolus, then 5-FU 2400 mg/m^2 as a 46-h infusion, biweekly] or FOLFIRI-3 (irinotecan 100 mg/m^2 on day 1 and leucovorin 400 mg/m^2, then 5-FU 2400 mg/m^2 as a 46-h infusion and irinotecan 100 mg/m^2 repeated on day 3, biweekly) after failure of gemcitabine and platinum-based chemotherapies as a systematic policy in two institutions between January 2005 and May 2010. Tumor response, time to progression (TTP), overall survival rate (OS) and grade 3-4 toxicities were retrospectively studied. Subgroup analyses were performed to search for prognostic factors.

RESULTS: Sixty-three patients (52.4% male, median age 59 years) were analyzed. Among them, 42.9% were PS 0, 38.1% were PS 1 and 19.0% were PS 2. Fifty one patients (81.0%) had liver metastases. Before the FOLFIRI regimen, patients had received 1 line (n = 19), 2 lines (n = 39) or 3 lines (n = 5) of chemotherapy. Median TTP obtained with the line before FOLFIRI was 3.9 mo (95% CI: 3.4-5.3 mo). A total of 480 cycles was completed (median: 6 cycles, range: 1-51 cycles). The main reason for discontinuing FOLFIRI was tumor progression (90.3%). Tumor control was achieved in 25 patients (39.7%) (partial response: n = 5, stable disease: n = 20) with FOLFIRI. Median TTP was 3.0 mo (95% CI: 2.1-3.9 mo) and median OS was 6.6 mo (95% CI: 5.3-8.1 mo). Dose adaptation was required in 36 patients (57.1%). Fifteen patients (23.8%) had grade 3-4 toxicities, mainly hematological (n = 11) or digestive (n = 4). Febrile neutropenia occurred in 3 patients. There was no toxic death. PS 2 was significantly associated with poor TTP [hazard ratio (HR): 16.036, P < 0.0001] and OS (HR: 4.003, P = 0.004).

CONCLUSION: The FOLFIRI regimen had an acceptable toxicity and an interesting efficacy in our study, limited to patients in good condition (PS 0-1).

Key words: Pancreatic cancer; Pancreatic adenocarcinoma; Metastases; Chemotherapy; 5-fluorouracil; Irinotecan; Camptothecin; FOLFIRI regimen

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INTRODUCTION

Pancreatic adenocarcinoma (PAC) accounts for 2%-3% of all cancers but is the fourth leading cause of cancer death in Western countries[1]. More than 80% of patients present with unresectable disease and most of those with operable tumors who undergo resection have local relapse or metastases[2]. The overall prognosis of metastatic PAC remains poor, with a 5-year survival rate of less than 5%[3].

Gemcitabine became the reference regimen as first-line chemotherapy in patients with metastatic PAC after a randomized trial showed significant improvement in median overall survival (OS) compared with 5-fluorouracil (5-FU) (5.6 mo *vs* 4.4 mo, $P = 0.002$)[4]. Over the past decade, multiple phase II and III studies have attempted to improve these results using various combinations of gemcitabine with other agents but no significant benefit on survival has been found compared with gemcitabine alone, except for erlotinib which resulted in a modest but significant improvement in OS (6.2 mo *vs* 5.9 mo, $P = 0.038$)[5,6]. A phase III trial comparing the FOLFIRINOX regimen (folinic acid/5-FU, irinotecan and oxaliplatin combination) to gemcitabine as first-line treatment for metastatic PAC showed that this combination was superior to gemcitabine (OS: 11.1 mo *vs* 6.8 mo, $P < 0.001$)[7].

In clinical practice, about half of metastatic PAC patients with disease progression under gemcitabine treatment remain in acceptable clinical condition and thus may receive subsequent line(s) of chemotherapy. A retrospective series of 117 patients evaluated the feasibility and benefits of second- and third-line chemotherapies in patients with metastatic PAC after the failure of gemcitabine[8]. Fifty three (45%) received two lines and 24 (21%) received three or more lines. Median time to progression (TTP) and OS from the beginning of the second line were 2.3 mo and 4.7 mo, respectively. The FFCD 0301 phase III trial was the first randomized study to evaluate a chemotherapy strategy with a second line of treatment in the treatment plan. It compared the combination of folinic acid/5-FU and cisplatin followed by gemcitabine or the reverse sequence in metastatic PAC[9]. The second line of therapy was administered at disease progression to 68% of patients who received folinic acid/5-FU and cisplatin as a first line treatment and to 55% in the gemcitabine arm (non-significant). Median progression-free survival (PFS) and OS in the two arms were not significantly different. Although there is no standard regimen in this setting, two randomized studies have indicated that the combination of folinic acid/5-FU and oxaliplatin appeared to be superior to both best supportive care (4.9 mo *vs* 2.3 mo, $P = 0.008$) and folinic acid/5-FU alone (6.0 mo *vs* 3.0 mo, $P = 0.014$) as a second line of treatment[10]. Other regimens have been tested in non-randomized phase II studies, but the samples were small and information on World Health Organization (WHO) performance status (PS) and disease stage were often lacking[11].

Preclinical studies have shown that the camptothecin analog irinotecan has significant activity in both cultured pancreatic tumor cells and in xenograft models[12,13]. Irinotecan monotherapy in previously untreated PAC patients yielded response rates (RR) of 9%-27%[14-16]. *In vitro* studies have suggested that there is a synergistic effect between irinotecan and 5-FU[17-19]. A multicenter phase II study with folinic acid/5-FU and irinotecan day 1/day 3 combination (FOLFIRI-3 regimen) showed promising activity in chemotherapy-naive patients with locally advanced or metastatic PAC, with a median PFS and OS of 5.6 mo and 12.1 mo, respectively, and a manageable toxicity profile[20]. A randomized phase II study has compared modified FOLFIRI-3 and a modified FOLFOX schema (folinic acid/5-FU and oxaliplatin combination) as second-line chemotherapy in that setting[21]. The efficacy was similar, with 6-mo OS rate of 27% and 30%, respectively. An Italian group reported a retrospective series of 40 patients with gemcitabine-resistant locally advanced or metastatic PAC treated with a standard FOLFIRI (FOLFIRI-1, folinic acid/5-FU and irinotecan day 1 combination) regimen[22]. Median TTP was 3.7 mo and median OS was 6 mo.

Because no data exist on the efficacy of the FOLFIRI regimen after the failure of both gemcitabine and platinum salts, we performed a retrospective study to evaluate the efficacy and safety of this regimen in patients with advanced PAC in that setting. As locally advanced PAC may have a more favorable natural history than metastatic PAC, we decided to exclude locally advanced PAC patients from the study to have a homogeneous population.

MATERIALS AND METHODS

Patients

All patients with histologically confirmed, metastatic PAC, after failure (progression or major toxicity) of gemcitabine and platinum-based chemotherapies, re-

ceived an irinotecan-based regimen as a systematic policy after discussion during a weekly multidisciplinary meeting in our institutions (Saint Antoine Hospital, Paris and Beaujon Hospital, Clichy), if they met the following criteria: previous treatment with gemcitabine and platinum salt (combined or given in consecutive lines); WHO PS $\leq$ 2; at least one bidimensionally measurable lesion according the Response Evaluation Criteria in Solid Tumors (RECIST); absence of severe uncontrolled cardiovascular, metabolic, infectious or renal disease; serum bilirubin level < 1.5 times the upper limit of normal; polynuclear neutrophil count > 1500/mm^3; platelet count > 100 000/mm^3.

Chemotherapy regimen

The FOLFIRI-1 regimen consisted of irinotecan 180 mg/m^2 administered as a 90-min infusion on day 1, together with leucovorin 400 mg/m^2 for 2 h followed by an 5-FU 400 mg/m^2 bolus, then a 46-h infusion of 5-FU 2400 mg/m^2. The FOLFIRI-3 regimen consisted of irinotecan 100 mg/m^2 administered as a 60-min infusion on day 1, together with leucovorin 400 mg/m^2 for 2 h, then a 46-h infusion (without bolus administration) of 5-FU 2400 mg/m^2 and irinotecan 100 mg/m^2 repeated on day 3 at the end of 5-FU infusion. Only FOLFIRI-3 (intensified) regimen has been evaluated in phase II studies in PAC[20,21]. However, the FOLFIRI-1 regimen is extensively used in clinical practice for treatment of other gastrointestinal cancers and seems to be less toxic. Thus, the choice between the FOLFIRI-1 or FOLFIRI-3 regimen was left up to the discretion of the investigator. The chemotherapy cycles were repeated every two weeks if the clinical and biochemical assessment was compatible (as mentioned above).

Patients who developed a cholinergic syndrome received preventive treatment with atropine (0.25 mg subcutaneously) during all subsequent cycles. Late-onset diarrhea was treated using high-dose loperamide. When severe neutropenia occurred and/or did not recover to grade $\leq$ 1 on day 14, a granulocyte-colony stimulating factor was given.

The irinotecan and the 5-FU dosages were reduced by 20% when any grade 3-4 toxicity occurred; other dose adjustments were decided on an individual basis. Treatment was stopped when the tumor progressed or severe toxicity occurred, or at the patient's request. Further treatments are discussed on an individual basis.

Assessment of therapeutic efficacy

Treatment efficacy was assessed on a clinical evaluation, carbohydrate antigen (CA) 19-9 serum levels and thoraco-abdominal computed tomography (CT). Assessment of treatment efficacy was performed every 2 mo (four cycles) or earlier in patients with clinically suspected progression. Tumor response was assessed using CT according to RECIST[23]. A complete response (CR) was defined as complete disappearance of all assessable disease, partial response (PR) as a decrease of > 30% in the sum of the largest diameters of target lesions, stable disease (SD) as a decrease of < 30% or an increase of < 20% in measurable lesions, and progressive disease (PD) as an increase of > 20% in measurable lesions or the appearance of new malignant lesions. Patients who were not assessable by CT but who presented clinical and/or biochemical (CA 19-9 serum level elevation) evidence of disease progression or who died from a cancer-related cause were also considered as PD. The sum of CR, PR and SD was reported as the tumor control rate (TCR). The sum of CR and PR was reported as overall RR (ORR). OS was defined as the time from the first day of the FOLFIRI regimen to the date of death (all causes) or last follow-up. TTP was defined as the time from the first day of the FOLFIRI regimen to the date of disease progression. Patients without progression were censored at the last follow-up.

Safety

Toxicity was assessed before each cycle with the National Cancer Institute Common Toxicity Criteria (version 3.0). A complete physical examination was performed and a full blood count and serum bilirubin, aminotransferases, alkaline phosphatase and creatinine assays were obtained before each treatment cycle.

Data collection

The following patient data were collected and analyzed retrospectively: age; gender; primary tumor location; stage at the time of diagnosis; previous surgery and/or radiotherapy; previous lines of chemotherapy; TTP with the previous line; reasons for stopping previous line; presence or absence of liver metastases at the beginning of FOLFIRI regimen; PS at the beginning of FOLFIRI regimen; type of FOLFIRI regimen (FOLFIRI-1 or FOLFIRI-3); number of cycles administered; best tumor response; grade 3-4 toxicities; dose adaptation; TTP and OS from the beginning of FOLFIRI regimen; reasons for stopping FOLFIRI regimen; further treatments.

Statistical analysis

All analyses were performed using Stata software (version 11.0; StataCorp). All statistical tests were two sided with an alpha type one error of 5%. TTP and OS were estimated using the Kaplan-Meier method and described using median or rate of TTP/OS at a specific time point with 95% CI. Log-rank tests were used to compare survival curves.

Univariate and multivariate Cox proportional hazard model analyses were performed with the following variables: stage at diagnosis; previous treatment by radiotherapy; number of previous chemotherapy lines; presence or absence of liver metastases; PS at the beginning of FOLFIRI regimen.

For exploratory purposes, subgroup analyses were performed according to the following variables: primary tumor location; stage at the diagnosis; previous treatment by surgery or radiotherapy; number of previous

Table 1 Characteristics of the 63 patients with metastatic pancreatic cancer treated with FOLFIRI after failure of gemcitabine and platinum-salts

Characteristics	Data
Age (yr)	
Median	59 (range: 24-81)
Sex (%)	
Male	33 (52.4)
Performans status (%)	
PS 0	27 (42.9)
PS 1	24 (38.1)
PS 2	12 (19.0)
Liver metastases (%)	
Present	51 (81.0)
Number of previous lines before FOLFIRI (%)	
1	19 (30.2)
2	39 (61.9)
≥ 3	5 (7.9)

chemotherapy lines; presence or absence of liver metastases; PS at the beginning of FOLFIRI regimen; type of FOLFIRI regimen (FOLFIRI-1 or FOLFIRI-3).

RESULTS

Patients

Between January 2005 and May 2010, 63 patients with metastatic PAC fulfilled the criteria for this study. Their characteristics are shown in Table 1. Median age was 59 years (range: 24-81 years). Thirty three patients (52.4%) were male. The primary tumor was located in the head of the pancreas in 32/50 patients (64.0%). Twenty three patients (36.5%) had undergone prior surgery and 16 patients (25.4%) had received chemoradiotherapy. Twenty-seven patients (42.9%) were WHO PS 0, 24 patients (38.1%) were PS 1 and 12 patients (19.0%) were PS 2 at the beginning of the FOLFIRI regimen. Fifty one patients (81.0%) had liver metastases.

Before receiving the FOLFIRI regimen, patients had received one line (gemcitabine-oxaliplatin: n = 19, 30.2%), two lines (gemcitabine then FOLFOX regimen: n = 39, 61.9%) or three lines (n = 5, 7.9%) of chemotherapy. The previous line had been stopped for tumor progression in 55 patients (87.3%) and due to toxicity (oxaliplatin-related neuropathy) in the remaining 8 patients (12.7%).

Study treatment and drug delivery

Fifty five patients (87.3%) received the FOLFIRI-1 regimen and 8 patients (12.7%) received the FOLFIRI-3 regimen. A total of 480 cycles was completed (median: 6 cycles per patient, range: 1-51 cycles per patient).

The reasons for discontinuing the FOLFIRI regimen was progression in 56/62 patients (90.3%), toxicity in one patient (febrile neutropenia: n = 1, 1.6%), a tumor control ≥ 6 mo or at the patient's request in 4 patients (6.5%), surgery in one patient (1.6%) who had a major response. Sixteen patients (25.4%) who remained in good condition at the time of FOLFIRI withdrawal received a subsequent line following the multidisciplinary proposal.

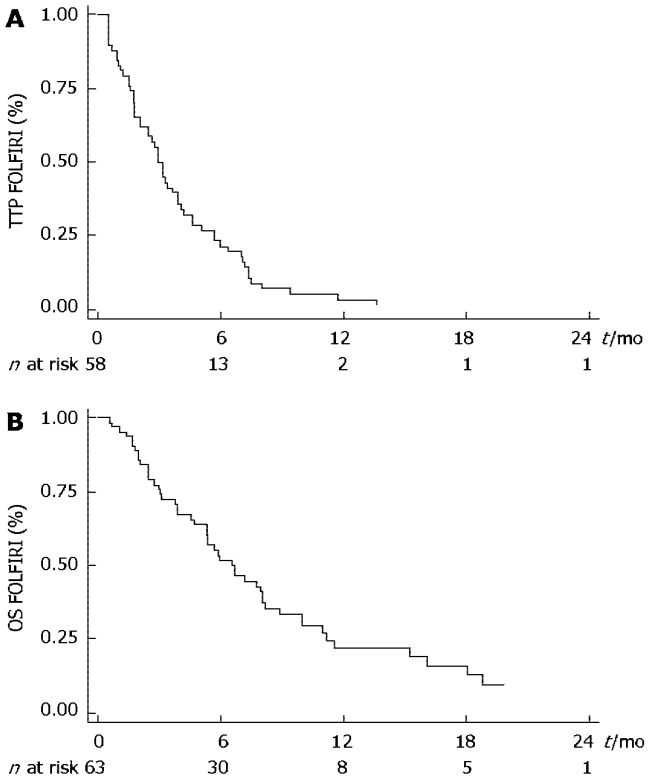

Figure 1 Time to progression (A) and overall survival (B) from the beginning of FOLFIRI for the 63 patients. TTP: Time to progression; OS: Overall survival.

Tumor response and survival

Median TTP obtained with the last line of chemotherapy before FOLFIRI was 3.9 mo (95% CI: 3.4-5.3 mo). Tumor control was obtained with the FOLFIRI regimen in 25 patients (39.7%) (CR: n = 0; PR: n = 5, 7.9%; SD: n = 20, 31.8%). ORR was 7.9% (5/63). Median TTP was 3.0 mo (95% CI: 2.1-3.9 mo) and median OS was 6.6 mo (95% CI: 5.3-8.1 mo) (Figure 1A and B).

Subgroup analysis

WHO PS was the only variable that was significantly associated with TTP and OS (Tables 2 and 3). Median TTP was 4.2 mo (95% CI: 3.2-7.0 mo) in PS 0 patients, 3.0 mo (95% CI: 1.8-4.1 mo) in PS 1 patients and 0.7 mo (95% CI: 0.5-1.5 mo) in PS 2 patients (Figure 2A). Median OS after the beginning of FOLFIRI was 8.2 mo (95% CI: 6.7-11.0 mo) in PS 0 patients, 5.4 mo (95% CI: 3.0-16.1 mo) in PS 1 patients and 2.5 mo (95% CI: 0.7-3.1 mo) in PS 2 patients (Figure 2B). PS 2 was significantly associated with a poor TTP [hazard ratio (HR) = 16.036, $P < 0.0001$] and OS (HR = 4.003, $P = 0.004$) in univariate analysis and in multivariate analysis also. No significant association was found between other variables and survival, except for the number of previous lines with TTP in multivariate analysis (Tables 2 and 3).

Dose adaptation and safety

Dose adaptation was required in 36 patients (57.1%).

Table 2 Results of univariate and multivariate analysis for time to progression with FOLFIRI

	Progression	Univariate analysis			Multivariate analysis		
	Yes/no (57/1)	HR	95% CI	*P* value	HR	95% CI	*P* value
Primary tumor location[1]				0.687			
Body or tail	18/0	1					
Head	28/1	0.883	(0.483-1.615)				
Stage at the diagnosis				0.666			0.652
Resectable	22/0	1			1		
Locally advanced	15/1	1.235	(0.631-2.418)		1.394	(0.662-2.939)	
Metastatic	20/0	0.905	(0.490-1.674)		1.299	(0.626-2.695)	
Previous surgery				0.659			
No	37/1	1					
Yes	20/0	0.882	(0.506-1.539)				
Previous radiotherapy				0.411			0.168
No	44/0	1			1		
Yes	13/1	1.305	(0.692-2.459)		1.770	(0.786-3.987)	
Number of previous chemotherapy lines				0.284			0.026
1	16/0	1			1		
2	36/1	0.649	(0.350-1.204)	0.170	0.385	(0.193-0.770)	
3	5/0	1.128	(0.411-3.096)	0.814	0.554	(0.177-1.735)	
Liver metastases				0.531			0.564
No	9/0	1			1		
Yes	48/1	0.793	(0.383-1.640)		1.298	(0.535-3.151)	
WHO performans status				< 0.0001			< 0.0001
0	24/0	1			1		
1	22/1	1.325	(0.731-2.399)		1.431	(0.745-2.748)	
2	11/0	16.036	(5.926-43.394)		29.255	(9.278-92.248)	
Type of FOLFIRI regimen				0.124			
FOLFIRI-1	50/0	1					
FOLFIRI-3	7/1	0.503	(0.210-1.207)				

HR: Hazard ratio; WHO: World Health Organization. [1]Data available in 47 out of 58 cases.

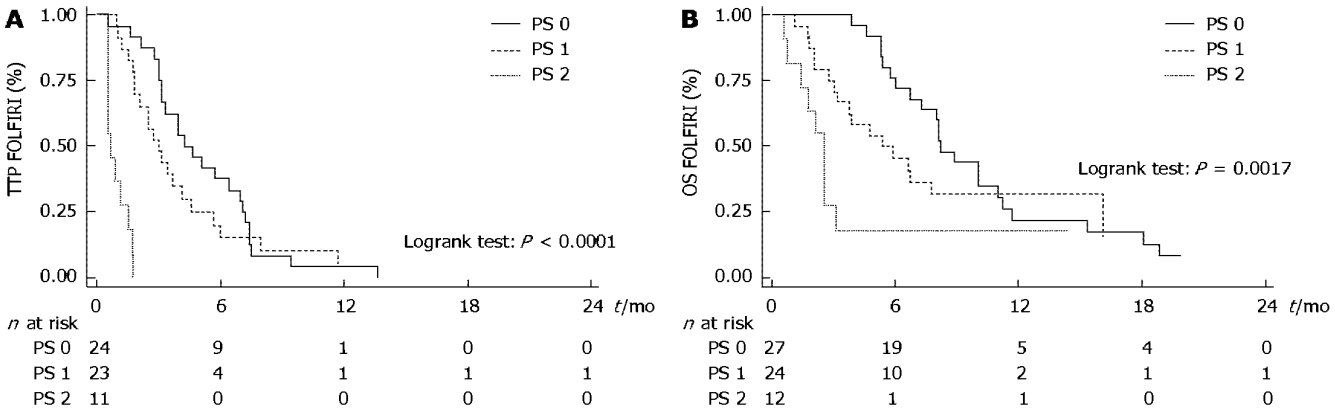

Figure 2 Subgroup analysis according to the World Health Organization performance status. A: Time to progression (TTP) according to World Health Organization (WHO) performance status (PS); B: Overall survival (OS) according to WHO PS.

The initial dose was reduced in 20 patients (31.7%) (19 patients who received the FOLFIRI-1 regimen and one patient who received the FOLFIRI-3 regimen) for the following reasons: cholestasis (n = 11), PS 2 (n = 8) or age > 75 years (n = 2), pre-existent diarrhea (n = 2) or mucositis (n = 1), and an episode of grades 3-4 hematological toxicity during previous chemotherapy (n = 1). A subsequent reduction was proposed in 19 patients (30.2%) (18 patients who received the FOLFIRI-1 regimen and one patient who received the FOLFIRI-3 regimen). Fifteen (23.8%) of these patients had grade 3-4 toxicities, mainly hematological (n = 11, 17.5%) and/or digestive with diarrhea and/or mucositis (n = 4, 6.3%). Febrile neutropenia occurred in 3 patients (4.8%). There were no related deaths.

DISCUSSION

We have evaluated the efficacy and safety of the FOLFIRI regimen after the failure of both gemcitabine and platinum salts in 63 patients with metastatic PAC treated in two centers. Tumor control was obtained in 39.7% of

Table 3 Results of univariate and multivariate analysis for overall survival with FOLFIRI

	Death	Univariate analysis			Multivariate analysis		
	Yes/no (49/14)	HR	95% CI	*P* value	HR	95% CI	*P* value
Primary tumor location[1]				0.306			
Body or tail	16/2	1					
Head	25/7	0.717	(0.380-1.355)				
Stage at the diagnosis				0.754			0.644
Resectable	20/4	1			1		
Locally advanced	13/6	1.012	(0.497-2.058)		0.790	(0.346-1.806)	
Metastatic	16/4	1.271	(0.643-2.515)		1.205	(0.577-2.514)	
Previous surgery				0.203			
No	31/9	1					
Yes	18/5	0.676	(0.370-1.236)				
Previous radiotherapy				0.916			0.935
No	38/9	1			1		
Yes	11/5	0.964	(0.491-1.894)		0.965	(0.412-2.260)	
Number of previous chemotherapy lines				0.102			0.171
1	14/5	1			1		
2	30/9	1.234	(0.649-2.346)		1.197	(0.564-2.538)	
3	5/0	3.188	(1.090-9.326)		2.945	(0.930-9.324)	
Liver metastases				0.599			0.957
No	9/3	1			1		
Yes	40/11	1.217	(0.586-2.528)		0.978	(0.444-2.159)	
WHO performance status				0.004			0.004
0	22/5	1			1		
1	18/6	1.360	(0.719-2.573)		1.284	(0.650-2.535)	
2	9/3	4.003	(1.770-9.056)		4.702	(1.883-11.741)	
Type of FOLFIRI regimen				0.856			
FOLFIRI-1	42/13	1					
FOLFIRI-3	7/1	1.083	(0.458-2.562)				

HR: Hazard ratio; WHO: World Health Organization. [1]Data available in 50 out of 63 cases.

patients. The median TTP was 3.0 mo and the median OS after the beginning of FOLFIRI was 6.6 mo. Toxicity was frequent with the FOLFIRI regimen (grade 3-4 toxicities in 23.8% of patients, mainly hematological and digestive) but manageable as only one patient had to stop treatment. In the subgroup analysis, the WHO PS was the only variable that was significantly associated with TTP (HR = 16.036, $P < 0.0001$) and OS (HR = 4.003, $P = 0.004$). Patients with WHO PS 2 may not benefit from this regimen.

Irinotecan-based chemotherapies have previously been tested in advanced PAC. Irinotecan was tested as a single agent in the first line setting in three phase Ⅱ trials with interesting results, showing an ORR of 9%-27% and a median OS of 5.2-7.3 mo[14-16]. However, two phase Ⅲ trials that tested irinotecan combined with gemcitabine as first-line chemotherapy did not show a significant benefit in TTP (2.8-3.5 mo) and OS (6.4-6.6 mo), despite a higher response rate than with standard gemcitabine (ORR: 15%-16%)[24,25]. A randomized phase Ⅱ study confirmed that the antitumoral activity of the combination of gemcitabine and irinotecan is similar to a regimen of fixed dose gemcitabine, gemcitabine/cisplatin or gemcitabine/docetaxel[26]. Thus, the gemcitabine/irinotecan combination does not seem to be synergistic. In contrast, the efficacy of the combination of irinotecan and 5-FU has been shown to be interesting with acceptable toxicity (Table 4)[20,27-29]. This is supported by *in vitro* and *in vivo* data showing synergy between these two drugs[17-19]. These regimens have not yet been tested in a phase Ⅲ trial compared with gemcitabine. Recently, a phase Ⅲ trial comparing the FOLFIRINOX regimen (folinic acid/5-FU, irinotecan and oxaliplatin combination) to gemcitabine as first-line treatment for metastatic PAC showed a significant improvement in survival with the FOLFIRINOX regimen with a median PFS and OS of 6.8 mo and 11.1 mo, respectively[7]. Toxicity was significant (grade 3-4 in 54% the patients) but manageable, and no toxic death occurred. Patients included in this study were in good condition (WHO PS 0-1). In addition, an absence of cholestasis was required for inclusion which probably explains the unusually high rate of body/tail tumor localization.

Irinotecan as a single agent has been shown to be a well-tolerated but marginally effective regimen in gemcitabine-pretreated patients. ORR was less than 10% and median OS did not exceed 4-6.6 mo[30,31]. The results with irinotecan-based combination regimens in gemcitabine-resistant advanced PAC were conflicting (Table 4)[32-37]. Data on irinotecan and 5-FU combination regimens in this setting are scarce. A randomized phase Ⅱ study evaluated modified FOLFIRI-3 *vs* modified FOLFOX (folinic acid/5-FU and oxaliplatin combination) in patients with gemcitabine-resistant advanced PAC. Efficacy was comparable with both regimens with a 6-mo OS rate of 27% (95% CI: 13%-46%) and 30% (95% CI: 15%-49%), respectively[21]. An Italian group reported a retrospective series of 40 patients with gemcitabine-

Table 4 Phase I - II studies of regimens as first and second lines chemotherapy in advanced pancreatic cancer

Regimen	Number of patients	TCR/ORR (%/%)	TTP or PFS (mo)	OS (mo)
Phase I - II studies[1]				
Irinotecan/gemcitabine/5-FU[27]	30	43/7	3.4	8.3
G-FLIP (Irinotecan/gemcitabine/5-FU/cisplatin)[28]	31	68/26	6.1	8.1
FOLFIRI-3 (Irinotecan/5-FU)[29]	40	65/38	5.6	12.1
Irinotecan/S1[29]	16	75/44	4.9	11.3
Phase II studies[2]				
Irinotecan/raltitrexed[32]	19	53/16	4.0	6.5
IROX (Irinotecan/oxaliplatin)[33]	30	33/10	4.1	5.9
IROX (Irinotecan/oxaliplatin)[34]	14	50/21	1.4	4.1
G-FLIP (Irinotecan/gemcitabine/5-FU/cisplatin)[35]	34	44/24	3.9	10.3
Irinotecan/docetaxel[36]	14	21/0	1.2	4.4
MDI (Irinotecan/mitomycin/docetaxel)[37]	15	20/0	1.7	6.1

[1]Phases I - II studies of irinotecan and 5-fluorouracil (5-FU)-based regimens as first line chemotherapy; [2]Phase II studies of irinotecan-based regimens as second line chemotherapy. TCR: Tumor control rate; ORR: Overall response rate; TTP: Time to progression; PFS: Progression free survival; OS: Overall survival.

resistant locally advanced or metastatic PAC treated with the standard FOLFIRI-1 regimen[22]. As in our series, most patients were PS 0-1 (82.5%); 17.5% of patients had locally advanced PAC, while all patients in our series were metastatic. The efficacy was quite similar to our series: TCR: 50%, ORR: 15%, median TTP: 3.7 mo and median OS: 6 mo. In contrast, toxicity was higher, with 27% and 32% of grades 3-4 hematological and digestive toxicities respectively. Toxicity was more frequent in PS 2 patients (71%) than in PS 0-1 patients (45%). The difference in the incidence of severe toxicity between the two series was not due to a difference in the proportion of PS 2 patients (17.5% *vs* 19%). One explanation might be that the initial dose was frequently adapted in our series (31.7%), particularly in PS 2 patients (8/12, 66.7%). In contrast, there was no tumor control at 6 mo in a study using a combination of irinotecan and fluoropyrimidine (mostly capecitabine) in 34 patients, most of whom where PS 0-1, and only 6% of patients were alive 1 year after the beginning of this chemotherapy regimen[38]. Most patients (97%) had been pretreated with capecitabine. This suggests that the different dose intensity and administration schedule for fluoropyrimidine, as in the XELIRI regimen, and the synergy of capecitabine and irinotecan could not overcome possible acquired resistance to this drug.

Our current study is the largest retrospective series on chemotherapy with a combination of irinotecan and 5-FU in metastatic PAC after the failure of gemcitabine and platinum salts. It was a bi-center study with a homogenous population (metastatic, not locally advanced, PAC) and selection bias was reduced by the systematic treatment policy in both centers. However, patients were heterogeneous regarding the number of previous lines of chemotherapy or the type of FOLFIRI regimen. We could not compare the efficacy of the two FOLFIRI regimens due to the unequal distribution of patients between the two groups, with only 8 patients receiving the FOLFIRI-3 regimen. Moreover, this study included selected patients treated in high volume centers with teams that were experienced in the management of pancreatic tumors and their complications. Endoscopic procedures for the treatment of jaundice, a classic exclusion criteria for irinotecan (40%-60% of PAC), were easily accessible. Another possible bias was the high rate of previous surgery (32.9%). The natural history of operated patients might be more favorable than that of patients with unresectable PAC at diagnosis. The toxicity of the FOLIFIRI regimen was manageable, although dose adaptation was required in more than half of patients (57.1%). Only patients who are PS 0-1 seem to benefit from this regimen.

In conclusion, the FOLFIRI regimen is a valuable option in patients with metastatic PAC after failure of gemcitabine and platinum salts but should be considered for patients in good condition (WHO PS 0-1). Further studies are needed to determine whether FOLFIRI is a valuable option as first line therapy in advanced PAC.

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COMMENTS

Background

Pancreatic adenocarcinoma (PAC) is the fourth leading cause of cancer death in the Western countries. The overall prognosis of metastatic PAC remains poor with a 5-year survival rate of less than 5%. Gemcitabine is the reference first-line regimen for metastatic PAC treatment. About half of patients with metastatic PAC whose disease progresses under gemcitabine are eligible for subsequent line(s) of chemotherapy and there is no standard regimen in that setting. Preclinical and clinical studies have suggested that the combination of 5-fluorouracil (5-FU) and irinotecan (FOLFIRI regimen) may be beneficial in PAC. The research aimed to evaluate the efficacy and safety of FOLFIRI regimen in patients with metastatic PAC after the failure of gemcitabine and platinum salts.

Research frontiers

Tumor response rate, toxicity of irinotecan-based regimen, time to progression and overall survival were determined for the FOLFIRI regimen.

Innovations and breakthroughs

This is a homogeneous study of consecutive metastatic PAC patients treated by two experienced teams in the management of patients with pancreatic cancer. The present paper suggests that whereas the combination of gemcitabine and irinotecan was not effective enough, that of 5-FU and irinotecan appears to be beneficial regarding both efficacy and tolerability. In addition, the study series

provides original data about the appropriate target population for the irinotecan-based regimen, particularly taking into account the PS status.

Applications

Recently, the FOLFIRINOX schema, combining 5-FU, irinotecan and oxaliplatin, was shown to be superior to gemcitabine in a first-line setting, with an overall survival of 11.1 mo (95% CI: 9-13.1 mo) *vs* 6.8 mo (95% CI: 5.5-7.6 mo, hazard ratio = 0.57) ($P < 0.0001$), respectively, in selected patients (performance status 0-1, absence of cholestasis). However, due to the hematological toxicity of this combination, many patients are not eligible for first-line therapy. The sequence FOLFOX then FOLFIRI (or the reverse) may be an alternative and should be considered as being better tolerated.

Terminology

For treatment purposes, pancreatic tumors are generally classified as resectable, locally advanced, or metastatic. A locally advanced pancreatic cancer is a tumor involving the arterial axis (celiac trunk, mesenteric artery) and thus is not resectable despite no detectable metastases. This form of cancer should be distinguished from metastatic tumors as the prognosis is different (slightly better, and some patients can have surgical treatment in case of a good tumor response after chemotherapy) and separate analyses are needed. Thus, locally advanced PAC patients were excluded from the study.

Peer review

This is an interesting study in which authors evaluate the efficacy and safety of this regimen in patients with metastatic PAC after the failure of gemcitabine and platinum salts. The results are convincing and suggest that FOLFIRI regimen had an acceptable toxicity and an interesting efficacy in our study, limited to patients in good condition.

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Clinical Studies

A randomised phase II study of modified FOLFIRI.3 *vs* modified FOLFOX as second-line therapy in patients with gemcitabine-refractory advanced pancreatic cancer

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BACKGROUND: Only a few clinical trials have been conducted in patients with advanced pancreatic cancer after failure of first-line gemcitabine-based chemotherapy. Therefore, there is no current consensus or the treatment of these patients. We conducted a randomised phase II study of the modified FOLFIRI.3 (mFOLFIRI.3; a regimen combining 5-fluorouracil (5-FU), folinic acid, and irinotecan) and modified FOLFOX (mFOLFOX; a regimen combining folinic acid, 5-FU, and oxaliplatin) regimens as second-line treatments in patients with gemcitabine-refractory pancreatic cancer.

METHODS: The primary end point was the 6-month overall survival rate. The mFOlFIRI.3 regimen consisted of irinotecan (70 mg m^{-2}; days 1 and 3), leucovorin (400 mg m^{-2}; day 1), and 5-FU (2000 mg m^{-2}; days 1 and 2) every 2 weeks. The mFOLFOX regimen was composed of oxaliplatin (85 mg m^{-2}; day 1), leucovorin (400 mg m^{-2}; day 1), and 5-FU (2000 mg m^{-2}; days 1 and 2) every 2 weeks.

RESULTS: Sixty-one patients were randomised to mFOLFIRI.3 ($n=31$) or mFOLFOX ($n=30$) regimen. The six-month survival rates were 27% (95% confidence interval (CI) = 13–46%) and 30% (95% CI = 15–49%), respectively. The median overall survival periods were 16.6 and 14.9 weeks, respectively. Disease control was achieved in 23% (95% CI = 10–42%) and 17% patients (95% CI = 6–35%), respectively. The number of patients with at least one grade 3/4 toxicity was identical (11 patients, 38%) in both groups: neutropenia (7 patients under mFOLFIRI.3 regimen *vs* 6 patients under mFOLFOX regimen), asthaenia (1 *vs* 4), vomiting (3 in both), diarrhoea (2 *vs* 0), and mucositis (1 *vs* 2).

CONCLUSION: Both mFOLFIRI.3 and mFOLFOX regimens were tolerated with manageable toxicity, offering modest activities as second-line treatments for patients with advanced pancreatic cancer, previously treated with gemcitabine.

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Pancreatic cancer accounts for 3% of all cancers, but is the fifth leading cause of cancer death in Western countries (Yeo *et al*, 2005). At the time of diagnosis, approximately half of the patients have metastases, and the median survival time barely exceeds 6 months, whereas approximately one-third of patients diagnosed with locally advanced disease have median survival times ranging between 6 and 9 months. Thus, a small proportion of patients are eligible for surgery, the only curative treatment option, at diagnosis (Bilimoria *et al*, 2007). Even with surgery, prognosis remains poor; the 5-year overall survival was only 23.4% for patients undergoing pancreatectomy (Sener *et al*, 1999).

Although 5-fluorouracil (5-FU)-based chemotherapy has been reported to be superior to best supportive care alone (Palmer *et al*, 1994; Glimelius *et al*, 1996), and a pivotal phase III trial showed that gemcitabine offers a survival advantage over a weekly bolus infusion of 5-FU, accompanied by an improved clinical benefit (Burris *et al*, 1997), the overall therapeutic results are still disappointing; the response rate was 5.4% with a clinical benefit response rate of 23.8% and a 1-year survival rate of 18% in patients treated with gemcitabine.

Therefore, a number of clinical studies have been undertaken to enhance the effectiveness of front-line chemotherapy. Despite promising results in early-phase clinical studies, the majority of newer approaches have failed to show clinically meaningful therapeutic advantages over the standard infusion of gemcitabine alone. Although regimens consisting of gemcitabine in combination with erlotinib or capecitabine have shown statistically significant increases in survival duration, the small amount of survival benefit and accompanying toxicities result in difficulties related to their translation into clinically meaningful improvements (Cunningham *et al*, 2005; Moore *et al*, 2007).

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Considering the poor response rate (20% or less) of gemcitabine-based doublet treatment in the first-line setting, the short progression-free survival (PFS) (<4 months), and the increased use of gemcitabine as adjuvant treatment (Oettle *et al*, 2007), an additional problem in the therapeutic management of this common malignant disease, is the need for effective treatment alternatives in patients failing to respond to gemcitabine-based chemotherapy. To date, few studies have assessed second-line chemotherapy, primarily because of poor prognosis (Nakachi *et al*, 2007) and because of the limited life expectancy of those with advanced pancreatic cancer after failure of first-line chemotherapy (Kozuch *et al*, 2001; Tsavaris *et al*, 2005; Kulke *et al*, 2007; Xiong *et al*, 2008; Novarino *et al*, 2009). There is, therefore, a growing unmet need for a second-line chemotherapy regimen to treat patients with gemcitabine-refractory pancreatic cancer (Boeck and Heinemann, 2008; Kang and Saif, 2008).

The clinical benefit and safety of the FOLFIRI and FOLFOX regimens have been well established in a study of gastrointestinal cancer patients (Tournigand *et al*, 2004). In several phase II trials, irinotecan-based and oxaliplatin-based regimens have shown modest activity against advanced pancreatic cancer. A French group has reported that the FOLFIRI.3 regimen, composed of a split irinotecan infusion on days 1 and 3, with 5-FU for 2 days, showed promising activity in chemotherapy-naive and pre-treated patients with advanced pancreatic cancer. The confirmed response rate was 37.5%, with a median PFS of 5.6 months (Taieb *et al*, 2007). The study also suggested that there was no cross-resistance between gemcitabine and FOLFIRI.3 regimen. Furthermore, an oxaliplatin and 5-FU combination, at various doses and schedules, has been evaluated as second-line chemotherapy in pancreatic cancer patients after gemcitabine failure (Tsavaris *et al*, 2005; Gebbia *et al*, 2007; Novarino *et al*, 2009). Recently, a German group has reported that the 5FU/folinic acid (FA) plus oxaliplatin (OFF) regimen could prolong survival and improve the quality of life of advanced pancreatic cancer patients after gemcitabine failure compared with best supportive care alone with or without 5FU/FA (FF) (Oettle *et al*, 2005; Pelzer *et al*, 2008).

On the basis of these results, we conducted a randomised phase II study of the modified FOLFIRI.3 (mFOLFIRI.3) and modified FOLFOX (mFOLFOX) regimens as second-line treatments in patients with gemcitabine-refractory pancreatic cancer. The aim of this study was to select a better regimen, which should be investigated in future studies.

MATERIALS AND METHODS

Patients

Patients at least 18 years of age with histologically confirmed, locally advanced, or metastatic pancreatic adenocarcinoma, who were previously treated with gemcitabine-based first-line chemotherapy were eligible for this study if they met the following inclusion criteria: Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–2; measurable disease based on Response Evaluation Criteria in Solid Tumors (RECIST) criteria; no previous second-line chemotherapy; adequate bone marrow function, defined as a condition with leukocyte count >4000 per μl, absolute neutrophil count >1500 per μl, haemoglobin >9.0 g per 100 ml, platelets >100 000 per μl; adequate renal and hepatic function, defined as a condition with serum creatinine <1.5 mg per 100 ml, bilirubin <1.5 mg per 100 ml (<2.5 mg per 100 ml in patients with obstructive jaundice and adequately decompressed bile duct obstruction), and serum transaminase <three-fold the upper normal limit (<five-fold the upper normal limit for patients with liver metastasis); adequate nutritional status, defined as a condition with albumin >3.0 g per 100 ml; and the giving of written informed consent. Patients were excluded if they had histology indicating a condition other than adenocarcinoma, brain metastasis, significant gastrointestinal bleeding or obstruction, any serious co-morbidity, axial skeletal radiotherapy within 6 months before study commencement, or peripheral neuropathy of grade 2 or worse. This study was initially approved by the Institutional Review Board of the Asan Medical Center. The study was conducted according to the tenets of the Declaration of Helsinki and guidelines on good clinical practice. The clinical trial registration number was NCT00786006.

Study design and randomisation

This was an open-label, single-centre, randomised phase II trial using the two treatment arms of mFOLFIRI.3 and mFOLFOX. Random assignment was performed at a 1:1 ratio and patients were stratified by age (≤65 years *vs* >65 years), ECOG PS (0–1 *vs* 2), and an earlier best overall response to gemcitabine (non-disease progression *vs* disease progression).

Treatment dose and schedule

The mFOlFIRI.3 regimen consisted of irinotecan 70 mg m^{-2} (over 1 h) on day 1, leucovorin 400 mg m^{-2} (over 2 h) on day 1, 5-FU 2000 mg m^{-2} (over 46 h) from day 1, and irinotecan 70 mg m^{-2} (over 1 h) at the end of the 5-FU infusion every 2 weeks. The mFOLFOX regimen was composed of oxaliplatin 85 mg m^{-2} (over 2 h) on day 1, leucovorin 400 mg m^{-2} (over 2 h) on day 1, and 5-FU 2,000 mg m^{-2} (over 46 h) every 2 weeks. When haematologic or non-haematologic toxicities of grade ≥2 occurred, chemotherapy was delayed until recovery to grade ≤1. The doses of subsequent schedules were reduced by 25% in patients with grade ≥3 haematologic and non-haematologic toxicities, and if toxicity was considered to be attributable, by the attending physician, to only one drug; the doses of other drugs were not modified. Treatment was continued until the occurrence of disease progression, unacceptable toxicity, or patient's refusal to continue. If disease progression was observed and patient performance was good, crossover to the alternate treatment arm was permitted.

Pre- and on-treatment evaluation

Within 2 weeks before study enrolment, patients gave a complete medical history; underwent a full physical examination including ECOG PS; were sampled for a complete blood count, serum chemistry with electrolyte levels, a coagulation battery, and carbohydrate antigen 19–9 (CA 19–9) level; underwent urinalysis; underwent a chest X-ray; were assessed by electrocardiography; and were evaluated by computed tomography of the abdomen and pelvis (chest or any other region, if metastasis was suspected or previously detected). Before the administration of each cycle of chemotherapy, each patient was examined and reviewed for complete and differential blood counts and serum chemistry. More frequent review and monitoring were performed if clinically indicated. Tumour response was assessed every three cycles according to the RECIST criteria (Therasse *et al*, 2000). For each of these assessments, similar imaging techniques as used at baseline were used. The National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0, was used to assess toxicity.

Statistical analysis

The primary end point was the 6-month survival rate. The randomised two-arm phase II design was used to select the more promising regimen of the two in terms of this criterion (Simon *et al*, 1985). Using this design, the regimen with the better survival rate is selected, irrespective of the difference between protocols. To permit at least a 90% probability of selecting a truly better regimen when the absolute difference in the 6-month survival rate was 15%

or greater, 29 evaluable patients were needed in each arm. Survival time was calculated from the date of randomisation to the date of death from any cause. The secondary end points were overall response rate, PFS, overall survival (OS), and toxicity. Overall response rate was analysed on an intention-to-treat basis. PFS was defined as the time from randomisation to disease progression or death from any cause. PFS was censored at the date of the last visit for those patients who were alive without documented disease progression. OS and PFS were estimated by the Kaplan–Meier method. Patients were considered assessable if they had received at least two cycles of chemotherapy (over 4 weeks) and had at least one follow-up imaging study. However, patients were also considered assessable if they received less than two cycles because of rapid tumour progression. Survival curves were compared by the log-rank test. In multivariate analysis, Cox's proportional hazards model was used to identify independent prognostic factors for PFS and OS. All tests were two-sided and a P-value <0.05 was considered to be statistically significant. SPSS version 14.0 (SPSS, Chicago, IL, USA) was used for statistical analysis.

RESULTS

Patient characteristics

From January 2007 to December 2008, 61 pancreatic cancer patients were enrolled at the Asan Medical Center, Seoul, Korea; 31 were randomly assigned to the mFOLFIRI.3 arm and 30 to the mFOLFOX arm. One patient in the mFOLFIRI.3 arm withdrew consent after the first cycle of chemotherapy and was lost to follow-up. Baseline characteristics were well balanced between the two treatment arms (Table 1). The median patient age was 55 years (range 35–73 years) and all but one patient was of ECOG PS 0 or 1. Twenty-one patients (34%) had undergone previous surgery and two (3%) had received palliative radiotherapy. Of the 16 patients who were prescribed adjuvant chemotherapy, gemcitabine was administered to three patients. Gemcitabine plus capecitabine was given to most patients (75%). After disease progression to a stage at which a salvage regimen was required, a crossover to the alternate protocol was undertaken by 12 patients (39%) in the mFOLFIRI.3 arm and by 7 (23%) in the mFOLFOX arm. The median time to crossover to the alternate treatment was 8.3 weeks (range 3.3–18.1 weeks) in the mFOLFIRI.3 arm, and 15 weeks (range 7.0–32.6 weeks) in the mFOLFOX arm.

Primary end points

A total of 98 cycles of the mFOLFIRI.3 and 93 cycles of the mFOLFOX regimens were delivered with a median of 3 cycles (range 1–12 and 1–10 cycles, respectively) in both arms. With a median follow-up period of 24.4 weeks (range 0.8–40.8 weeks), 50 of 61 patients (82%) died. The 6-month survival rate was 27% in the mFOLFIRI.3 arm (95% confidence interval (CI) = 13–46%) patients and 30% for those in the mFOLFOX arm (95% CI = 15–49%). Except for two patients who died because of treatment-related complications, all deaths were attributable to disease progression *per se*.

Secondary end points

The overall response rate values are listed in Table 2. Response evaluation was possible in 28 patients in the mFOLFIRI.3 arm and in 26 patients in the mFOLFOX arm. In the mFOLFIRI.3 arm, two patients could not be evaluated because of early death, and were lost to follow-up before the first response evaluation. In the mFOLFOX arm, response evaluation could not be achieved in four patients because of early death (two patients), loss to follow-up (one patient), and patient's refusal to continue with the trial (one patient). The overall response rate in the intention-to-treat population was 7% in the mFOLFOX arm (95% CI = 1–22%). Overall response could not be ascertained in the mFOLFIRI.3 arm. The disease control rate (PR and stable disease) was 23% in the mFOLFIRI.3 arm (95% CI = 11–40%) and 17% in the mFOLFOX arm (95% CI = 7–34%).

Table 1 Patient characteristics

Characteristic	mFOLFIRI.3 ($n=31$) No. of patients (%)	mFOLFOX ($n=30$) No. of patients (%)
Age, median (range)	55 (37–73)	55 (35–69)
<60 years	19 (61)	18 (60)
⩾60 years	12 (39)	12 (40)
Gender		
Male	24 (77)	20 (67)
Female	7 (23)	10 (33)
ECOG PS		
0	5 (16)	5 (17)
1	26 (84)	24 (80)
2	0 (0)	1 (3)
Metastatic site		
Liver	19 (61)	21 (70)
Peritoneum	19 (61)	11 (37)
Lung	6 (19)	5 (17)
Lymph nodes	15 (48)	14 (47)
Others	9 (29)	5 (17)
Prior treatment		
Surgery	10 (32)	11 (37)
Palliative radiotherapy	1 (3)	1 (3)
Adjuvant chemotherapy	7 (23)	9 (30)
Neoadjuvant chemoradiotherapy	0 (0)	1 (3)
Prior gemcitabine-based regimen		
Gemcitabine	4 (13)	2 (7)
Gemcitabine/capecitabine	20 (64)	26 (86)
Gemcitabine/erlotinib	4 (13)	2 (7)
Gemcitabine/cisplatin	3 (10)	0 (0)
Previous response to gemcitabine-based regimen		
CR	0 (0)	1 (3)
PR	10 (32)	9 (30)
SD	11 (35)	13 (43)
PD	10 (32)	7 (23)
Survival at analysis		
Alive	6 (20)	5 (17)
Dead	25 (81)	25 (83)
Crossover to alternative regimen	12 (39)	7 (23)

Abbreviations: ECOG PS = Eastern Cooperative Oncology Group performance status; CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease.

Table 2 Overall response rate

Overall Response	mFOLFIRI.3 No. of patients (%, 95% CI)	mFOLFOX No. of patients (%, 95% CI)
PR	0 (0, 0–10)	2 (7, 1–22)
SD	7 (23, 11–40)	3 (10, 3–26)
PD	21 (68, 49–83)	21 (70, 52–84)
Not evaluable	3 (10, 3–26)	4 (13, 5–30)
Disease control	7 (23, 11–40)	5 (17, 7–34)

Abbreviations: PR = partial response; SD = stable disease; PD = progressive disease.

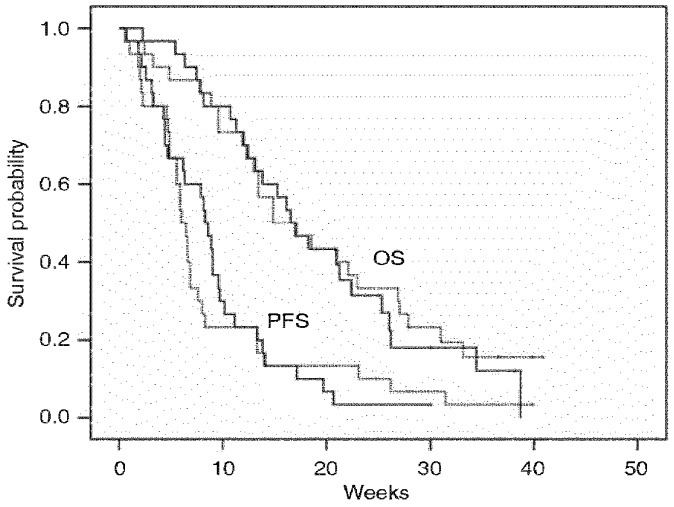

Figure 1 Survival curves for progression-free survival (PFS) and overall survival (OS). Modified FOLFIRI.3 (a regimen combining 5-fluorouracil, folinic acid, and irinotecan) is depicted as solid lines and modified FOLFOX (a regimen combining folinic acid, 5-FU, and oxaliplatin) as dotted lines.

The median PFS was 8.3 weeks for patients treated with mFOLFIRI.3 (95% CI = 6.9–9.6 weeks) and 6.0 weeks for those given mFOLFOX (95% CI = 5.1–6.9 weeks) (Figure 1). The median OS was 16.6 weeks for patients treated with mFOLFIRI.3 (95% CI = 12.5–20.6 weeks) and 14.9 weeks for those given mFOLFOX (95% CI = 8.0–21.8 weeks) (Figure 1). Turning to survival outcomes from the commencement of first-line chemotherapy, the median PFS was 34.9 weeks (95% CI = 30.8–38.9 weeks) and 37.0 weeks (95% CI = 32.0–42.0 weeks) for mFOLFIRI.3 and mFOLFOX, respectively. The median OS was identical at 47.1 weeks (95% CI = 39.0–55.2 weeks and 36.0–58.3 weeks, respectively).

Toxicity

The numbers of patients experiencing adverse events are presented in Table 3. In each treatment arm, 29 patients were available for toxicity assessment, and only two patients in the mFOLFOX arm were free from adverse events. The prevalence of severe toxicities was the same between the two regimens (38%); however, grade 3/4 asthaenia (3% *vs* 14%) developed more frequently in patients receiving mFOLFOX, whereas grade 3/4 diarrhoea (7% *vs* 0%) was more common in patients prescribed mFOLFIRI.3. Treatment-related mortality occurred in one patient in each group. One patient in the mFOLFIRI.3 arm died of septic shock complicated by febrile neutropaenia after 2 weeks of the first cycle. In one patient in the mFOLFOX arm, early death after the first cycle of chemotherapy was caused by severe pneumonia.

Prognostic factors

In a univariate analysis of survival outcomes according to the clinical variables of all 60 patients (gender, age, ECOG PS, hypoalbuminaemia, anaemia, resectability at initial diagnosis, liver metastasis, and PFS under gemcitabine), hypoalbuminaemia ($\leqslant 3.5$ mg 100 ml^{-1}) and ECOG PS $\geqslant 1$ were significant prognostic factors for poor PFS and OS. In multivariate analysis, however, only hypoalbuminaemia predicted poor PFS ($P = 0.02$, hazard ratio = 1.97, 95% CI = 1.14–3.39), but not OS.

DISCUSSION

Pancreatic cancer is well known to be refractive to chemotherapy and to show rapid progression. Until recently, patients with pancreatic cancer after gemcitabine-based chemotherapy failure have had little opportunity to receive second-line chemotherapy because of rapid performance deterioration (Nakachi *et al*, 2007; Kang and Saif, 2008). Therefore, few studies have focused on patients with advanced pancreatic cancer in a second-line setting. Moreover, as gemcitabine is known to be effective when used as adjuvant therapy, many patients who underwent curative resection received gemcitabine in this setting. This means that oncologists urgently require data on other chemotherapeutic options for gemcitabine-pretreated patients.

Gemcitabine plus oxaliplatin (GEMOX), oxaliplatin plus capecitabine (XELOX), capecitabine plus erlotinib, docetaxel plus gefitinib, and FOLFOX have been tested in gemcitabine-refractory pancreatic cancer patients and showed disease control rates of 19–53% and a median OS range of 2.9–6.7 months (Tsavaris *et al*, 2005; Demols *et al*, 2006; Kulke *et al*, 2007; Xiong *et al*, 2008; Brell *et al*, 2009; Novarino *et al*, 2009). Recently, another oxaliplatin-based regimen, 5-FU/FA plus oxaliplatin (OFF), was shown to offer significantly improved survival compared with 5-FU/FA (FF) in a phase III trial (CONKO 003) (Pelzer *et al*, 2008). In this randomised trial, including 160 gemcitabine-pretreated patients with advanced pancreatic cancer, patients receiving OFF achieved a median PFS of 13 weeks ($P = 0.012$) and a median OS of 26 weeks ($P = 0.014$), compared with 9 and 13 weeks, respectively, for FF-treated patients. However, there is no current consensus on optimal second-line therapy for gemcitabine-refractory advanced pancreatic cancer (Boeck and Heinemann, 2008; Kang and Saif, 2008). Both FOLFIRI.3 and FOLFOX have shown modest activity as first-line and second-line chemotherapy regimens (Tsavaris *et al*, 2005; Gebbia *et al*, 2007; Taieb *et al*, 2007; Novarino *et al*, 2009). We were also of the view that neither regimen showed significant cross-resistance to gemcitabine-based protocols (Gebbia *et al*, 2007; Taieb *et al*, 2007).

The results of this trial show that both combination regimens showed favourable efficacy and toxicity profiles in gemcitabine-pretreated patients with advanced pancreatic cancer. The 6-month survival rates were 27 and 30% and disease control rates were 23% and 17%, in patients treated with mFOLFIRI.3 and mFOLFOX, respectively. Of the 12 patients whose disease was controlled by these regimens, disease stabilisation was previously achieved in nine patients in gemcitabine-based regimens. The median PFS and median OS were 8.3 weeks and 16.6 weeks in the mFOLFIRI.3 arm, and 6.0 weeks and 14.9 weeks in the mFOLFOX arm, respectively. These were in line with the survival data of several previous studies (Tsavaris *et al*, 2005; Gebbia *et al*, 2007; Novarino *et al*, 2009).

Toxicities related to both regimens were quite expectable and generally manageable. Patients with toxicities of grade 3 or worse constituted 38% of each treatment arm. Common toxicities of both regimens included anaemia, neutropenia, asthaenia, nausea, vomiting, and mucositis. In accordance with the known toxicities of both regimens, diarrhoea developed more frequently in mFOLFIRI.3 arm patients and neuropathy was more common in those in the mFOLFOX arm. Although half the patients treated with mFOLFOX experienced peripheral neuropathy, this was mostly of grade 1. This may be related to a lower cumulative dose of oxaliplatin because of the early dropout caused by rapid disease progression. However, treatment-related mortality occurred in patients prescribed either regimen, and hence physicians need to guard against infectious complications in patients treated with these protocols.

Turning to prognostic factors affecting PFS and OS, hypoalbuminaemia, implying poor nutritional status, was a poor prognostic factor for PFS in this study. In contrast to a previous study (Herrmann *et al*, 2008), we could not find an association between the time to progression under first-line chemotherapy ($\leqslant 6$ months) and PFS under second-line therapy, or residual survival. However, it is hard to draw conclusions with regard to this, because this study had small sample sizes, which might result in insufficient statistical power detecting significant prognostic factors.

Table 3 Treatment-related toxicities

	mFOLFIRI.3 no. of patients (%)			mFOLFOX no. of patients (%)		
Toxicity	**G 1-2**	**G 3-4**	**All G**	**G 1-2**	**G 3-4**	**All G**
Anaemia	14 (48)	1 (3)	15 (52)	15 (50)	1 (3)	16 (55)
Neutropenia	6 (20)	7 (24)	13 (45)	8 (27)	6 (20)	14 (48)
Thrombocytopenia	3 (10)	1 (3)	4 (14)	9 (31)	1 (3)	10 (34)
Febrile neutropenia		1 (3)	1 (3)		0 (0)	0 (0)
Alopecia	3 (10)	0 (0)	3 (10)	0 (0)	0 (0)	0 (0)
Asthaenia	17 (58)	1 (3)	18 (62)	22 (76)	4 (14)	26 (90)
Diarrhoea	10 (34)	2 (7)	12 (41)	5 (17)	0 (0)	5 (17)
Anorexia	5 (17)	1 (3)	6 (21)	6 (21)	2 (7)	8 (28)
Nausea	12 (41)	1 (3)	13 (45)	13 (45)	1 (3)	14 (48)
Vomiting	6 (20)	3 (10)	9 (31)	11 (38)	3 (10)	14 (48)
Mucositis	8 (27)	1 (3)	9 (31)	8 (28)	2 (7)	10 (34)
Neurotoxicity	1 (3)	0 (0)	1 (3)	13 (44)	0 (0)	13 (45)
Maximum/patients*	18 (62)	11 (38)		16 (57)	11 (38)	

Abbreviation: G = grade. *Maximum/patients, maximal toxicity in an individual patient. The numbers of patients experiencing adverse events are listed.

Clinical Studies

Although this trial used adequate primary and secondary outcomes to represent the characteristics of the two regimens, the lack of assessment of clinical benefit or quality of life is a limitation of our study.

In conclusion, our trial not only showed that both mFOLFIRI.3 and mFOLFOX regimens could be safely used but also showed modest anti-cancer activities in gemcitabine-pretreated patients. Although further clinical trials are necessary for comparison with other regimens, these protocols may be reasonable therapeutic options in a second-line setting for patients with advanced pancreatic cancer, who were previously treated with gemcitabine-based chemotherapy.

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FOLFIRI.3, a new regimen combining 5-fluorouracil, folinic acid and irinotecan, for advanced pancreatic cancer: results of an Association des Gastro-Entérologues Oncologues (Gastroenterologist Oncologist Association) multicenter phase II study

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Background: The purpose of the study was to prospectively evaluate the efficacy and tolerability of the FOLFIRI.3 regimen in patients with unresectable pancreatic adenocarcinoma.

Patients and methods: Chemotherapy-naive patients with histologically proven advanced pancreatic adenocarcinoma were treated with the FOLFIRI.3 regimen, consisting of irinotecan 90 mg/m^2 as a 60-min infusion on day 1, leucovorin 400 mg/m^2 as a 2-h infusion on day 1, followed by 5-fluorouracil (5-FU) 2000 mg/m^2 as a 46-h infusion and irinotecan 90 mg/m^2, repeated on day 3, at the end of the 5-FU infusion, every 2 weeks.

Results: Forty patients were enrolled, of whom 29 (73%) had metastatic disease. A total of 441 cycles were delivered (1–53). Grade 3–4 neutropenia occurred in 35% of the patients, accompanied by fever in two cases. Other relevant grade 3–4 toxic effects were nausea-vomiting (27%) and diarrhea (25%). Grade 2 alopecia occurred in 48% of the patients. There were no treatment-related deaths. The confirmed response rate was 37.5%. Stable disease was observed in 27.5% of the patients. The median progression-free and overall survivals were 5.6 months and 12.1 months, respectively. The 1-year survival rate was 51%.

Conclusion: The FOLFIRI.3 regimen seems to be active on advanced pancreatic cancer and to have a manageable toxicity profile. The lack of cross-resistance between FOLFIRI.3 and gemcitabine-based regimens allows efficient second-line therapies.

Key words: irinotecan, pancreatic cancer, systemic chemotherapy

introduction

Pancreatic cancer causes about 50 000 deaths annually in Europe and is the fourth leading cause of death by cancer in the Western countries; the mortality and incidence rates are similar [1–3]. The overall 6-month and 1-year survival rates among patients with advanced disease are, respectively, 35% and <10% in most studies [1–3]. About 80% of patients have unresectable or metastatic forms at diagnosis [4]. Systemic chemotherapy protocols for unresectable pancreatic cancer have given disappointing results during the last 20 years. Several drugs, given alone or in combination, have been tested in phase II and III trials, with objective response rates ranging from 0% to 20% and median survival times not exceeding 6 months [5]. One randomized trial showed the superiority of single-agent gemcitabine over single-agent 5-fluorouracil (5-FU) therapy and established gemcitabine as the reference for advanced pancreatic cancer [6]. The objective response rates, however, in large randomized trials of gemcitabine ranged from 4% to 16%, and the median survival time was only 4.6–6 months [7–10].

Irinotecan (Aventis, France), a camptothecin analogue, has a stronger growth-inhibiting effect than cisplatin, mitomycin C and fluorouracil on cultured pancreatic adenocarcinoma cells [11]. Irinotecan is also highly active on pancreatic tumor cells in culture and in xenograft models. [12, 13] Irinotecan monotherapy has been tested in patients with previously untreated pancreatic cancer, yielding response rates of 9%–27% [14, 15]. Second-line irinotecan monotherapy has also shown a degree of activity [16, 17]. In most trials, however, the response rates were low (<10%) and survival was poor.

In vitro studies indicate that synergism between irinotecan and 5-FU is sequence dependent, cytotoxicity being stronger when irinotecan is administered before 5-FU [18–20]. *In vivo*,

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however, a phase II randomized study of colorectal cancer patients indicated that cytotoxicity was stronger when irinotecan was administered after 5-FU [21]. These studies gave rise to the FOLFIRI.2 regimen, consisting of a simplified LV5FU2 administration, followed by irinotecan 180 mg/m^2 at the end of 5-FU infusion [22]. The latter phase II study, involving heavily pretreated colorectal cancer patients, showed encouraging efficacy but major toxicity. The same team subsequently designed a regimen (FOLFIRI.3) in which the irinotecan dose is administered in two halves, one before 5-FU and the other at the end of the 5-FU infusion. This regimen was then tested in a multicenter phase II study involving patients with metastatic colorectal cancer who had previously received FOLFOX. The response rate was 26% and the median progression-free and overall survival times were 5.1 and 10 months, respectively [23].

Here, in a multicenter phase II study, we evaluated the FOLFIRI.3 regimen in previously untreated patients with advanced pancreatic cancer.

patients and methods

patients

The following criteria were used for patient selection: histologically or cytologically proven pancreatic ductal adenocarcinoma; unresectable locally advanced or metastatic disease; at least one measurable lesion (response evaluation in solid tumors (RECIST) criteria); no previous chemotherapy or radiotherapy; age between 18 and 75 years; World Health Organization (WHO) performance status (PS) of less than three; initial morphologic assessment at least 3 weeks before treatment; adequate bone marrow status (polymorphonuclear neutrophils >1.5 g/l, platelets >100 g/l and hemoglobin >10 g/dl), renal function (serum creatinine level <125 μmol/l) and liver function [serum bilirubin level <1.5 × the upper limit of normal (ULN), alkaline phosphatase (ALP) and transaminase levels <3 × ULN] and estimated life expectancy >2 months. Surgical unresectability was observed during laparotomy or decided by a multidisciplinary staff meeting in each participating center. The study was approved by the Pitié Salpêtrière Hospital ethics committee, and written informed consent was obtained from each patient. Patients were fully informed of the type and modalities of the treatment, as well as possible adverse effects and expected benefits. The pretherapeutic work-up included a complete physical examination, WHO PS, body weight, symptoms, abdominal computed tomography (CT) scan, CA 19-9 assay, standard chest X-ray examination and, if required, thoracic CT scan.

the FOLFIRI.3 regimen

FOLFIRI.3 consists of irinotecan 90 mg/m^2 administered as a 60-min infusion on day 1, together with leucovorin 400 mg/m^2 over 2 h, 5-FU 2000 mg/m^2 administered as a 46-h infusion and irinotecan 90 mg/m^2 repeated on day 3, at the end of the 5-FU infusion (Figure 1). The chemotherapy cycles were repeated every 2 weeks if the polymorphonuclear neutrophil count was >1500/mm^3, the platelet count >100 000/mm^3 and the serum bilirubin level <1.5 × ULN.

The use of antiemetic prophylaxis was decided locally. Patients who developed a severe cholinergic syndrome received preventive treatment with atropine (0.25 mg subcutaneously) during all subsequent cycles. Patients who developed late-onset diarrhea received high-dose loperamide following specific guidelines. If severe neutropenia occurred and/or if neutropenia did not recover to grade 1 or 0 on day 14, a granulocyte colony-stimulating factor (G-CSF) could be used during subsequent cycles.

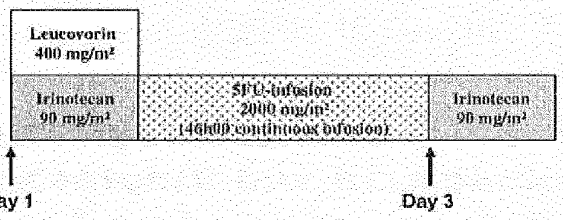

Figure 1. The FOLFIRI.3 regimen.

The irinotecan dosage was reduced to 80 mg/m^2 and the 5-FU dosage was reduced by 20% if grade 3–4 toxicity occurred; other dose adjustments were decided on an individual basis. Dose reescalation was not permitted.

Treatment was interrupted if the tumor progressed or severe toxicity occurred, and at the patient's request. Second-line chemotherapy with gemcitabine, oxaliplatin, 5-FU and cisplatin was offered if the chemotherapist considered it appropriate.

assessment of therapeutic efficacy and symptom relief

The primary end point for efficacy was the tumor response rate, defined as the sum of complete and partial responses based on the RECIST criteria [24]. Tumor responses were assessed by means of helicoidal CT every 2 months (four cycles) or earlier in patients with suspected progression. Complete responses were defined as complete disappearance of all assessable disease. Partial responses were defined as a decrease of >30% in the sum of the largest diameters of target lesions. Stable disease was defined as a decrease of <30% or an increase of <20% in measurable lesions. Progressive disease was defined as an increase of at least 20% in measurable lesions or the appearance of new malignant lesions.

A second CT scan was carried out 4 and 8 weeks after the first scan to confirm complete and partial responses. All CT scans for responder patients were reviewed by an external response review committee (ERRC), composed of two independent radiologists who were not otherwise involved in the study. Secondary end points for efficacy included the time to progression and the progression-free and overall survival times. Body weight, WHO PS and symptoms were recorded at the beginning of each chemotherapy session.

toxicity

Toxicity was assessed with the National Cancer Institute Common Toxicity Criteria (version 3.0). A full blood count was carried out each week to assess hematological toxicity, and the patients had a complete physical examination and serum bilirubin, transaminase, ALP and creatinine assays before each treatment cycle. The patients were interviewed before each session, focusing on pain, nausea, vomiting, mucositis, diarrhea, asthenia, weight loss and neurological disorders. All patients who received at least one treatment session were considered assessable for toxicity.

statistical analysis

The main purpose of this study being to assess the response rate to the FOLFIRI.3 regimen, Simon's two-stage method was used for statistical analysis [25]. The population size was calculated to demonstrate treatment efficacy for an objective response rate ≥30% and treatment inefficacy for an objective response rate ≤10%, with a 5% alpha risk and 90% power. At the end of the first phase (18 patients included), the trial was to be stopped for treatment inefficacy if the number of objective responses was zero or one. If more than one objective response was observed, the trial was to be continued until a total of 35 patients had been enrolled. Assuming that 15% of the patients would be inassessable, 40 patients needed to be

enrolled. All analyses have been carried out on intention-to-treat. The results are expressed as means ± standard deviation or as ranges, as appropriate. Follow-up started at the outset of treatment. The censoring event for responses was the start of disease progression. The censoring event for survival was the date of death. Overall and progression-free survivals were determined using the Kaplan–Meier method.

results

From June 2003 to June 2005, 40 patients with advanced pancreatic adenocarcinoma were enrolled by seven French centers participating in this prospective study. The patients' clinical features and laboratory findings are shown in Table 1. Median age was 58 years (range 42–74) and the male–female sex ratio was 1.67 (25 men and 15 women). Twenty-nine patients (73%) had metastatic disease. Twenty patients had undergone surgery before inclusion, seven for curative treatment and 13 for palliative treatment or exploration. Concerning the seven patients who underwent a previous curative surgery, they all relapsed within 3–12 months after surgery and five of them had more than one metastatic site at relapse. One patient with metastatic relapse had received external irradiation (45 Gy) >6 months before the study treatment was initiated. Thirty-nine patients were assessable for toxicity and 34 for the tumor response.

Table 1. Characteristics of the patients before treatment

	No. of patients (%)
General	
Enrolled	40
Measurable lesions	40
Assessable for response	34
Assessable for toxicity	39
Age, median (minimum–maximum), years	58 (42–74)
Male/female	25/15
WHO PS	
0	9 (26)
1	19 (40)
2	12 (34)
Pancreas tumor sites	
Head	18 (45)
Body	12 (30)
Tail	10 (25)
Disease stage	
Stage III/IVa	11 (27)
Stage IVb	29 (73)
Disease sites	
Pancreas	33 (82.5)
Liver	25 (63)
Lymph nodes	6 (15)
Peritoneum	4 (10)
Lung	4 (10)
Others	2 (5)
Prior treatment	
None	20 (47.5)
Surgery	20 (47.5)
Palliative radiotherapy	1 (2.5)
Adjuvant chemotherapy	1 (2.5)
Palliative chemotherapy	0 (0)
Initially symptomatic	27 (67)

WHO, World Health Organization; PS, performance status.

tumor responses and survival

Six objective responses were observed in the first 18 assessable patients, authorizing further recruitment. The overall results are shown in Table 2. Objective tumor responses were observed in 37.5% of the 40 patients [95% confidence interval (CI) 24% to 53%]. There was one complete response and 14 partial responses. Eleven patients (27.5%) had stable disease. Tumor progression occurred in eight patients (20%) and six patients (15%) were not assessable, mainly because death occurred before the first planned evaluation. Three patients were classified as responders by the investigators but not by the ERRC, who considered that the sum of the largest diameters of the target lesions had fallen by <30% (24%–28%). Finally,

Table 2. Efficacy results (n = 40)

	Assessed by the ERRC	Assessed by the investigators
Objective response rate		
No.	15	18
%	37.5	45
95% CI	24–53	30–60
Metastatic		
No.	12	12
%	41	41
95% CI	25–59	25–59
Locally advanced		
No.	4	7
%	36	63
95% CI	15–65	35–85
Duration of response (months)		
Median	9.1	10.2
95% CI	5.5–11.9	6.8–13.9
Progression-free survival (months)		
All		
Median	5.6	
95% CI	3.7–8.7	
Metastatic		
Median	4.8	
95% CI	3.7–9.4	
Locally advanced		
Median	6.4	
95% CI	5–9.1	
Overall survival (months)		
All		
Median	12.1	
95% CI	5.8–16.4	
Metastatic		
Median	12.1	
95% CI	5.2–17.9	
Locally advanced		
Median	10.3	
95% CI	5.2–13.8	

ERRC, external response review committee; CI, confidence interval.

the overall response rate was 41% (95% CI 25% to 59%) in metastatic patients and 36% (95% CI 15% to 65%) in patients with locally advanced disease.

With a median follow-up of 21.5 months, the mean progression-free and overall survival times were 5.6 and 12.1 months, respectively. The 1-year survival rate was 51% (Figure 2). As usually observed in this setting, median overall survivals were 12.1 (95% CI 5–12.1), 15.6 (95% CI 8–17.9) and 5.8 months (95% CI 4–10.3) in patients with WHO PS of zero, one and two, respectively.

Two patients underwent surgical resection of their tumor remnants. The first patient was treated for metachronous liver metastases ($n = 5$) and had a durable (2 year) major response (>90%) to the FOLFIRI.3 regimen. He underwent right hepatectomy followed by a further 6 months of FOLFIRI.3 and is still alive with no detectable disease 8 months after surgery. The second patient was treated for a single pathologically proven metachronous lung metastasis. She had a partial response lasting for 6 months, then underwent lobectomy of the right lung and received a further 3 months of adjuvant FOLFIRI.3. A metastasis appeared in the left lung 6 months later and the pulmonary resection was again carried out. She refused adjuvant chemotherapy, and is alive and free of detectable disease 6 months after the last surgical procedure.

PS improved in 16 (51%, 95% CI 35–68) of the 31 patients whose initial WHO PS was more than zero. Weight gain was observed in 50% of the patients with initial weight loss and initial signs such as pain, asthenia or anorexia declined in 14 (52%) of the 27 initially symptomatic patients. Median delay to symptom relief was 4 weeks.

Six patients were still being treated with the FOLFIRI.3 regimen at the time of the final analysis. Three patients with locally advanced disease were given concomitant radiochemotherapy after 8, 11 and 12 FOLFIRI.3 cycles. Another 22 patients were given second-line chemotherapy consisting of gemcitabine + oxaliplatin ($n = 13$), gemcitabine alone ($n = 6$) or 5-FU + cisplatin C ($n = 3$). Six patients received a third line of chemotherapy with 5-FU or gemcitabine.

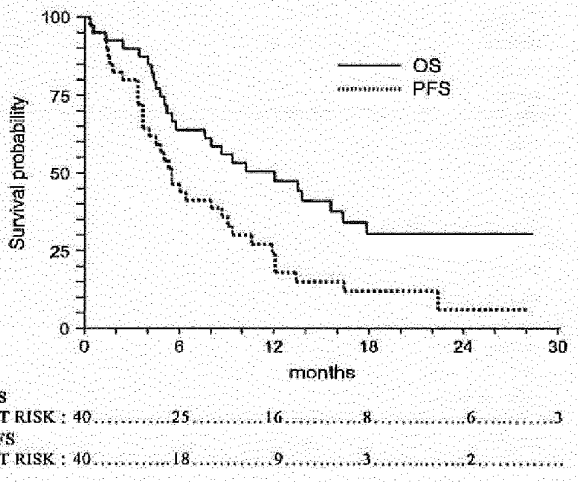

Figure 2. Overall survival (OS) and progression-free survival (PFS).

toxicity

Thirty-nine patients were included in the toxicity assessment (Table 3). A total of 441 chemotherapy sessions were administered, with a median of eight per patient (range 1–53). There were no treatment-related deaths. Fourteen patients (35%) developed grade 3–4 neutropenia. Grade 4 febrile neutropenia occurred in two patients who were not receiving growth factors. Five patients received G-CSF, for a total of 13 cycles. No new grade 3–4 toxic events were observed after cytotoxic dose reduction and/or G-CSF initiation.

Nonhematologic grade 3–4 toxic effects mainly consisted of gastrointestinal (GI) disorders. Despite routine prophylaxis with corticosteroids and setrons, grade 3 nausea-vomiting (considered to be one event) was the most frequent adverse effect, being observed in 11 patients (27%). Nausea-vomiting generally began 3 h after starting the infusion and lasted 1–3 days. Ten patients (25%) experienced grade 3 diarrhea, leading to hospitalization in two cases. All but one of the patients were able to continue treatment after a cytotoxic dose reduction and/or symptomatic treatment intensification. Aprepitant was necessary to control nausea and vomiting in three patients.

discussion

Until recently, pancreatic cancer was considered to be chemoresistant. This apparent chemoresistance was partly attributed to overexpression of the multidrug resistance and glutathione *S*-transferase genes in the normal and tumor-bearing pancreas [26, 27]. Despite disappointing results overall, chemotherapy has, over the last 10 years, improved the survival and quality of life of some patients with advanced pancreatic cancer [28]. 5-FU was widely used before 1997 to treat locally advanced and metastatic cancer of the pancreas [29]. In 1997, gemcitabine, which is easy to administer and well tolerated, was shown to be superior to 5-FU and became the new reference standard for this disease, although combinations based on platinum analogues and 5-FU are still widely used in France. Randomized trials of gemcitabine in combination with a second cytotoxic agent have failed to demonstrate any superiority over gemcitabine monotherapy, except for the gemcitabine plus capecitabine combination, but the final results of the two promising phase III studies are still awaited [7, 8, 10, 30–35].

Table 3. Toxicity

Toxicity (NCI-CTC)	Grade 1, N	Grade 2, N	Grade 3, N	Grade 4, N
Neutropenia	2	10	7	7
Thrombocytopenia	3	0	1	–
Anemia	10	19	3	–
Alopecia	12	19	–	–
Diarrhea	10	13	10	–
Nausea-vomiting	10	12	11	–
Mucositis	12	5	1	–
Neurotoxicity	5	1	–	–
Hand-foot syndrome	3	–	–	–
Maximum/patient (%)	5 (12.5)	10 (25)	18 (45)	7 (17.5)

NCI-CTC, National Cancer Institute Common Toxicity Criteria.
–, not observed.

Recently, the addition of anti-EGFR (epidermal growth factor) and anti-vascular endothelial growth factor to gemcitabine therapy was reported to yield response rates of 12%–21% and overall survival times of 7.1–8.8 months [36, 37]. Therefore, better systemic treatments using more efficient therapeutic regimens are still needed to treat advanced pancreatic cancer patients.

Irinotecan-based chemotherapies have previously been used for palliative treatment of pancreatic cancer [17, 31, 38–40]. The gemcitabine–irinotecan combination (IRINOGEM) gave promising results in phase II trials [40, 41], with objective response rates of 20%–25% and survival times of 5.7–7 months, but a subsequent phase III trial versus gemcitabine monotherapy gave negative results, with a response rate of only 16% and a median overall survival time of 6 months [31]. More recently, Conroy et al. [38] reported the results of a multicenter phase II trial testing 5-FU, oxaliplatin and CPT-11 combination therapy (FOLFIRINOX) in patients with locally advanced and metastatic pancreatic cancer. The objective response rate was 26%, as confirmed by an ERRC. The overall survival time was 10.2 months, the time to progression was 8.2 months and the median progression-free survival time is not given in the final publication.

We observed a 37.5% objective response rate in our trial. Furthermore, two of our patients with metachronous metastatic relapses were able to undergo secondary surgical R0 resection after long-lasting objective responses to the FOLFIRI.3 regimen. The tumor response is often difficult to assess in patients with locally advanced disease because of a frequent desmoplastic reaction around the organ. Major differences between the assessments of the investigators and the ERRC were observed in this subgroup, with three patients out of 11 classified as responders by the investigators and as stable by the ERRC. Moreover, the overall response rate was a little bit better in metastatic patients (41%) than in patients with locally advanced disease (36%). Concerning survival, it is noteworthy that the progression-free survival time was about half the overall survival time. Although second-line chemotherapy is classically considered ineffective on advanced pancreatic cancer, more than two-thirds of our patients received gemcitabine- or platinum-based second-line chemotherapy, and 45% of them had an objective response or disease stabilization. Thus, second-line chemotherapy with drugs showing no cross-resistance with the FOLFIRI.3 regimen might have improved the overall survival rate in this study. These results are in keeping with the trend in routine practice to offer further chemotherapy to patients with unresectable pancreatic cancer whose tumor progresses after first-line chemotherapy, as reported in other phase II and phase III trials [17, 39, 42–44]. Finally, although quality of life was not specifically assessed in this trial, about 50% of the patients gained weight, experienced symptom relief and had an improvement in their PS. These good results are not due to a patient selection bias, as about one-third of our patients had PS of two (WHO), one-quarter had more than two metastatic sites and five patients died before the first assessment of treatment efficacy. Thus, the objective response rate (37.5%, as confirmed by an ERRC) and the median overall survival time (12.1 months) observed in this study compare very favorably with the results of the latter two trials [31, 38] of irinotecan-based chemotherapies in pancreatic cancer.

The FOLFIRI.3 regimen has acceptable tolerability despite hematological and GI toxicity. These toxic effects were manageable in all the patients, and only 12.5% of patients had to stop the treatment because of severe adverse effects. No toxic deaths occurred. In future, however, patients with poor PS and other factors of poor prognosis such as a low albumin level, loss of appetite and high ALP or lactate dehydrogenase levels [7, 32, 45] may not be eligible for this regimen. Indeed, 50% of our patients with an initial PS of two experienced grade 3–4 neutropenia, 30% died before the first efficacy assessment and no tumor responses were observed in this subgroup of patients (only three had stable disease). Overall, 35% of the patients had grade 3 nausea-vomiting (taken as one event) and/or diarrhea. Only four of these patients had to be hospitalized for a few days and only one had to stop treatment of a GI adverse event. Concerning hematotoxicity, with grade 3–4 neutropenia in 35% of patients and only one case of grade 3 thrombocytopenia, the FOLFIRI.3 regimen seems to be more toxic than gemcitabine monotherapy but to be better tolerated than the FOLFIRINOX [38] and IRINOGEM regimens [31]. These results may be further improved by more frequent use of G-CSF prophylaxis.

In conclusion, with an objective response rate of 37.5%, a median overall survival time of 12 months and acceptable tolerability, the FOLFIRI.3 regimen seems to be active in patients with previously untreated advanced pancreatic cancer. The lack of cross-resistance between FOLFIRI.3 and gemcitabine-based regimens allowed efficient second-line therapy at treatment failure in this work. The FOLFIRI.3 regimen should now be tested in a randomized phase III trial versus gemcitabine.

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Phase I study of liposome encapsulated irinotecan (PEP02) in advanced solid tumor patients

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Abstract

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Background: PEP02 is a novel nanoparticle liposome formulation of irinotecan aiming to enhance tumor localization and improve pharmacokinetic properties of irinotecan and its active metabolite-SN38. The aims of the study are to define the dose-limiting toxicity (DLT), maximum tolerated dose (MTD) and pharmacokinetics (PK) of PEP02 in patients with advanced refractory solid tumors. **Methods:** Pts with advanced refractory solid tumors, ECOG PS 0–1, and adequate hematological, hepatic and renal functions were eligible. PEP02 was given as 90mins i.v. infusion, repeated every 3 weeks. The doses would have been escalated from 60, 120, 180 to 240 mg/m^2 in a single-patient cohort accelerated titration design. PK samples were collected on days 1, 2, 3, 8 and 21. **Results:** A total of 11 pts (M/F 1/10; median age 47, range 41–67) were enrolled onto three dose levels, with 1, 6 and 4 pts at dose level I (60 mg/m^2), II (120 mg/m^2) and III (180 mg/m^2), respectively. DLT was observed in 3 pts, including 1 at dose level II (grade 3 catheter-related infection) and 2 at dose level III (grade 3 diarrhea and febrile neutropenia in 1 and treatment-related mortality secondary to grade 4 diarrhea and neutropenia in 1). MTD was determined as 120 mg/m^2. The PK of total irinotecan after PEP02 dosing were characterized by, i.e. after 120 mg/m^2, low clearance (mean = 0.0591 L/m^2/hr), small volume of distribution (mean = 1.8 L/m^2, similar to plasma volume), and prolonged terminal half-life (mean=29.5 hr). The plasma concentration-time profiles of encapsulated irinotecan (PEP02) in each pt matched approximately with those of total irinotecan indicating

that the release of irinotecan from liposomes occurred slowly over time. Comparing with published PK parameters after 125 mg/m^2 of irinotecan, the Cmax of SN-38 after 120 mg/m2 of PEP02 was lower (9.2±3.5 vs 26.3±11.9 ng/mL), the terminal t1/2 of SN-38 was longer (75.4±43.8 vs 10.4±3.1 hrs) and the AUC of SN-38 was larger (710±395 vs 229±108 ng.h/mL). The best response of 10 evaluable pts was PR in 2 (cervical and pancreatic cancer) and SD in 3. **Conclusions:** The MTD of PEP02 monotherapy at 3-week interval is 120 mg/m^2, which will be the recommended dose for future phase II studies. Preliminary data suggest that PEP02 exhibits encouraging pharmacokinetic, safety and efficacy profiles.

No significant financial relationships to disclose.